Function of group I extensor feedback in the control of locomotion

by

Patrick J. Whelan

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy

Division of Neuroscience

Edmonton, Alberta

Fall 1996

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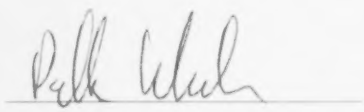

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Dedication

I wish to dedicate this thesis to my soulmate, Nancy.

Abstract

It is evident that sensory feedback plays an invaluable role in adjusting the basic locomotor pattern to the vigors and complexities of an ever changing environment. For example a chronic spinal cat can adjust its cadence to the speed of a moving treadmill by relying solely on sensory feedback from the moving limbs. It has been shown that afferent feedback can alter the timing of the step cycle in cats by changing the length of the extensor burst. The first part of my thesis work explored the effects of stimulation of extensor group I afferents (relaying input from muscle spindles and Golgi tendon organs) on the stance phase in walking cats. We discovered that stimulation of the group I afferents operating through a previously proposed oligosynaptic pathway could powerfully extend the stance phase and delay flexion in decerebrate cats. Similar but less robust effects were observed when comparable stimuli were applied to intact cats. One major question that arose was what factors could contribute to these differences between the stimulation of group I afferents in intact and decerebrate cats. We found from further work in the decerebrate walking cat that under certain conditions the effectiveness of the strong peripheral input from group I afferents could be modulated. In particular, increased stimulation of locomotor centres in the brainstem could decrease the efficacy of the group I stimulation. In addition to these studies we explored the possibility of plasticity in the locomotor-dependent group I excitatory pathway. We found that three to four days after cutting the LGS nerve in an otherwise intact cat stimulation of the synergistic medial gastrocnemius (MG) group I afferents would increase the duration of the extensor burst to a greater extent than similar stimulation of the contralateral MG nerve. Interestingly, the recovery of function in the intact cat after axotomy appeared to be correlated to an increase in the efficacy of the MG afferents. The recalibration of the MG group I pathway was influenced by supraspinal sites as transection of the spinal cord abolished the plasticity in many animals. In summary, my thesis work has shown that extensor afferent feedback can affect the timing and the amplitude of the extensor burst. These effects are dependent on the state of the animal, and the central pathways mediating this effect can be recalibrated in response to peripheral injury.

Acknowledgements

I would like to thank Keir Pearson for all his outstanding support over the last 3 years. His enthusiasm for science will always be an inspiration to me. Thanks are also due to my laboratory colleagues, Gordon Hiebert, Karim Fouad, and John Misiaszek, for their help, ideas and humor. I would like to thank Rod Gramlich for his excellent technical assistance. Over the last years I have benefited from working with Jaynie Yang, Arthur Prochazka, David Bennett and Monica Gorassini and I would like to thank each of them. The financial support provided by the Alberta Heritage Foundation for Medical Research and the Natural Sciences and Engineering Council of Canada is greatly appreciated. A special thank you to John Brooke and David Collins whose enthusiasm and love of their work helped guide me into graduate studies. Finally, I would like to thank my best friend Nancy for her support and love.

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List of Abbreviations

CPG	Central Pattern Generator
EMG	Electromyographic
ENG	Electroneurogram
IP	Iliopsoas
LG	Lateral gastrcnemius
LGS	Lateral gastrocnemius and Soleus
MG	Medial gastrocnemius
MLR	Mesencephalic Locomotor Region
St	Semitendinosis
TA	Tibialis Anterior
VL	Vastus lateralis
VL/VI	Vastus lateralis and Vastus intermedius
VM	Vastus medialis

Chapter 1

General Introduction[1]

It is evident that the reflex as well as other phenomena of the mammalian spinal cord present a large field of inquiry, being much more varied and extensive than previous experience had led us to suppose. (Sir Michael Foster, 1879; as cited in Liddell (1960)).

Every time we walk sensory receptors in our muscles are generating thousands of action potentials per second signaling various muscle parameters such as length, velocity and force. However surprisingly little is known about how these signals contribute to our walking pattern. The main goal of my doctoral research was to establish whether these signals could regulate the stepping pattern of a walking cat. Specifically we tested whether signals that arise from extensor muscle receptors that detect force (group Ib) and stretch (group Ia) of extensor muscles could alter the step cycle. The main sections of the thesis are outlined in figure 1-0. Chapter 2 of this thesis demonstrates that in a walking cat, activation of extensor group I afferents can prolong stance and delay the onset of swing. This suggests that when extensor muscle receptors that signal load are active the foot is prevented from leaving the ground, which would presumably ensure that swing is only initiated when it is safe to do so. Chapter 3 expands the results presented in chapter 2 (which used decerebrate walking animals) to the intact cat and demonstrates that similar activation of extensor group I afferents in the intact walking cat produces more variable effects on the stance to swing transition than in decerebrate walking animals. Finally, chapters 4 and 5 discuss the ability of the central nervous system to adjust the strength of the reflex pathways underlying the effects described above in response to axotomy of selected extensor nerves.

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[1] Parts of this chapter are currently in press. Whelan, P.J. Control of locomotion in the decerebrate cat. Prog.Neurobiol. 1996 (In Press)

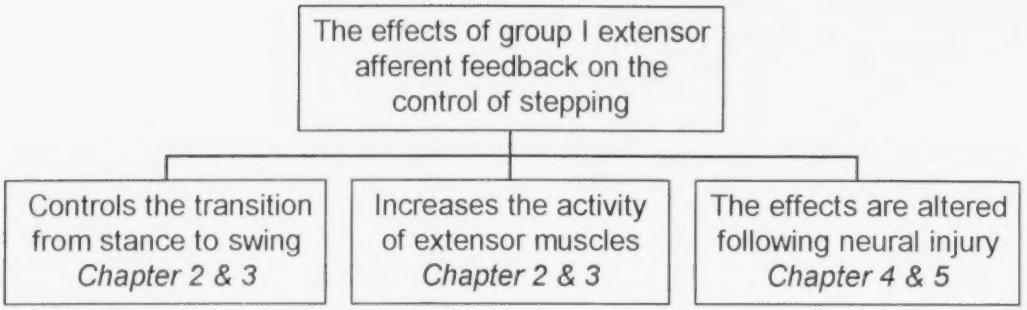
The effects of group I extensor afferent feedback on the control of stepping
Controls the transition from stance to swing
Chapter 2 & 3
Increases the activity of extensor muscles
Chapter 2 & 3
The effects are altered following neural injury
Chapter 4 & 5

Figure 1-0: The main sections of the thesis

The ideas for our experiments would not have been conceived if it had not been for the results of previous experiments which suggested that extensor group I afferents could play an important role in controlling locomotion (Duysens and Pearson, 1980; Conway et al., 1987). Therefore, before jumping into the body of the thesis I will outline the current knowledge of how afferent feedback is thought to *functionally* affect the walking pattern of cats. In the course of doing so I will point out how the work described in this thesis fits into the larger perspective of the afferent regulation of locomotion.

Afferent regulation of locomotion

Animals rarely move about in an unchanging environment. Fish, for example, must be able to maneuver and change course in response to currents, vegetation and rocks. Terrestrial animals face far more variability as they move across uneven terrain rife with its own dangers. To survive, animals must possess motor patterns that are robust yet flexible enough to handle unusual situations. Even during normal unperturbed locomotion the motor pattern must be able to sequence and coordinate the activity of many muscles to produce a single step (Rossignol, 1996). It is evident that evolution has crafted walking machines whose complexity we are only beginning to reveal. Progress in understanding locomotion in mammals such as the cat has

advanced at a steady pace over the last thirty years (for reviews see table 1-0). Yet many of the key concepts regarding the control of locomotion were advanced nearly 90 years ago by Sherrington (1910) and Graham-Brown (1922). These include the concepts that the isolated spinal cord is capable of producing the basic locomotor pattern and that reflex pathways can regulate its output. Today we know a great deal more about the anatomy and physiology of the spinal cord and sensory receptors, and the basic concepts advanced so long ago have been expanded using more sophisticated techniques and apparatus. However, there are still many gaps in our knowledge of how locomotion is controlled and regulated. One such gap is the role played by proprioceptive inputs from the muscles of the leg during walking.

Type of Review	**References**
General	Grillner, (1981); Rossignol, (1996); Wetzel and Stuart, (1976)
Interneuronal Circuitry	Jankowska (1992); Jankowska & Edgley, (1993); McCrea, (1992)
Spinal Cord (CPG)	Baev and Shimansky, (1992); Delcomyn, (1980); Eidelberg, (1981); Grillner and Wallen, (1985); Loeb, (1987); Sillar, (1991); Pearson, (1987)
The Role of Afferent Information	Baev et al., 1991; Duysens and Tax, (1993); Gossard and Hultborn, (1991); Loeb (1981); Murphy and Martin, (1993); Pearson, (1995; 1993); Pearson and Ramirez (1996); Prochazka, (1989; 1993; 1996); Rossignol et al., (1981; 1988); Rossignol and Drew, (1986); Whelan, (1996)
Cerebellum	Armstrong, (1986); Arshavsky et al., (1986); Arshavsky and Orlovsky, (1986)
Brainstem Regions (MLR/SLR/MRF)	Armstrong, (1986); Garcia-Rill, (1986; 1992); Inglis and Winn, (1995); Jordan et al., (1992); Jordan (1991; 1986); Mogenson, (1987; 1990); Mori (1987), Mori et al., (1992); Reese et al., (1995); Shik and Orlovsky, (1976); Sinnamon (1993); Skinner and Garcia-Rill, 1990
Motor Cortex	Drew (1991), Armstrong (1986; 1988)
Kinematics and Interlimb Coordination	Grillner, (1981); Halbertsma, (1983), Rossignol, (1996); Rossignol et al. (1993);Wetzel and Stuart (1976)
History	Adrian (1966); Hall (1837); Hinsey et al (1930); Liddell (1960); Wetzel and Stuart (1976); Graham-Brown, (1922); Sherrington, (1910)

Table 1-0: Reviews of the neural control of locomotion

The power of afferent feedback is exemplified by the finding that chronic cats with a transected spinal cord can recover the ability to walk when trained daily on a treadmill (Barbeau and

Rossignol, 1987) even in spinal kittens who did not walk before (Grillner, 1981). After three weeks the cats can easily adjust their gait to the speed of the treadmill. Since these animals have a transected spinal cord, only afferent feedback from the moving limbs can provide the information necessary to adjust the output of the central pattern generator (CPG). The adaptability of the motor pattern by afferent feedback is also revealed when a split-treadmill is used to drive each hindlimb of intact cats at different speeds. If one side is sped up in relation to the other, perfect coordination is maintained; the stance phase of the 'fast' side is decreased relative to the 'slow' side. These adjustments can be made not only in intact cats (Halbertsma, 1983) but also in decerebrate (Kulagin and Shik, 1970; Yanagihara et al., 1993) and low-spinal locomoting cats (Forssberg et al., 1980).

Modification of the step cycle is primarily achieved by changes to the stance phase. These changes can be manifested in two ways. Firstly, the amplitude of the extensor burst can be changed. For example, when a cat is walking uphill the EMG amplitude of the extensors is increased compared to walking on a flat surface, while the flexor bursts remain unaltered (Pierotti et al., 1989; see also Smith and Carlson-Kuhta, 1995). and (2) the timing of the step cycle can be altered. Consequently, when a cat speeds up, the length of the stance phase is reduced while the swing phase remains more or less the same (Grillner, 1981; Rossignol, 1996). From these observations it was proposed that afferent feedback from a moving limb may control aspects of extensor muscle activity during the stance phase. Another possible role of afferent feedback is to gauge the amount of propulsive force that needs to be generated under different environmental conditions. The substantial progress which has been made in identifying the types of afferents that contribute to the control of the amplitude and the duration of the extensor burst is reviewed in the next section and the afferent contribution to the timing of the extensor burst in the subsequent section.

Afferent reinforcement of the Ongong Step Cycle

When an animal is walking, there must be a sufficient activity in the extensor muscles to carry the weight of the animal. Before locomotion commences there is an increase in the level of postural tone in the extensor muscles which occurs by activation of descending pathways (Oka et al., 1993; Mori et al., 1978; for review see Mori et al., 1992). Afferent feedback from extensor muscles adds to the maintenance of this postural tone and ensures that it is adjusted, depending on the load carried by the limb. The concept that afferent feedback acts to reinforce activity in muscles has endured for over 30 years. In the decerebrate locomoting cat, Severin (1970) estimated by reversible inactivation of the gamma motoneuron axons (thus eliminating the fusimotor drive to the extensor muscle spindles) that 50% of the extensor EMG activity was produced by group Ia activity. In the human, Yang et al. (1991) have estimated that 30-60% of the EMG amplitude in the soleus muscle can be accounted for by monosynaptic excitation from group Ia afferents. In addition to the monosynaptic pathway, two oligosynaptic excitatory pathways have been discovered in the cat that can affect the extensor burst and likely receive convergent afferent input from both group Ia and Ib afferents in extensor muscles (see figures 1-1 & 1-2) (Gossard et al., 1994; Pearson and Collins 1993; Guertin et al., 1995a). These pathways produce excitatory postsynaptic potentials (EPSP) in extensor motoneurons that are phasically modulated. Studies from reduced preparations have demonstrated that input from extensor group I afferents that project onto this oligosynaptic pathway can reinforce the extensor burst.

In the cat, (Pearson and Collins, 1993) as in other species, (DiCaprio and Clarac, 1981; Bässler, 1983; 1986; Lacquaniti et al., 1991, Skorupski 1996; Skorupski and Sillar, 1986) there is a reflex reversal of input from the force detecting afferents. In the walking system of the cat, input from Golgi tendon organs (GTO) in extensor muscles onto extensor motoneurons is reversed from

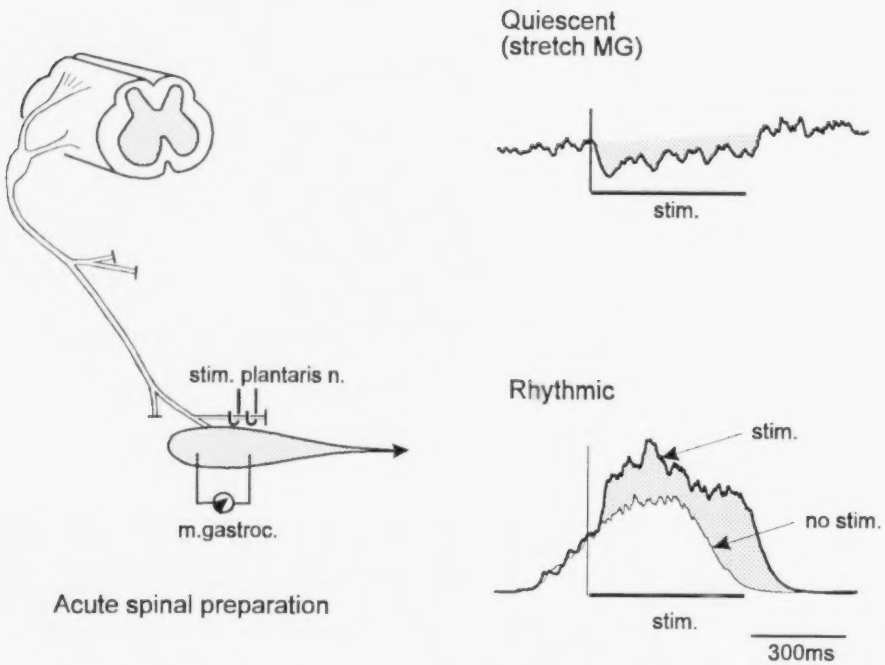

Figure 1-1: Reinforcement of the ongoing extensor burst in the acute spinal cat. The schematic figure on the left shows the methods used to show group Ib reflex reversal in the cat. Under quiescent conditions stimulation of the plantaris nerve would result in the classic Ib inhibition (Eccles et al., 1957a), however, under rhythmic conditions group I input would cause a significant excitation of the MG EMG at a latency of 30-40 ms. Note that since there are few monosynaptic inputs from PL to MG (Eccles et al., 1957b) most of the observed effects were mediated by oligosynaptic group I pathways (figures courtesy of Dr . K. Pearson).

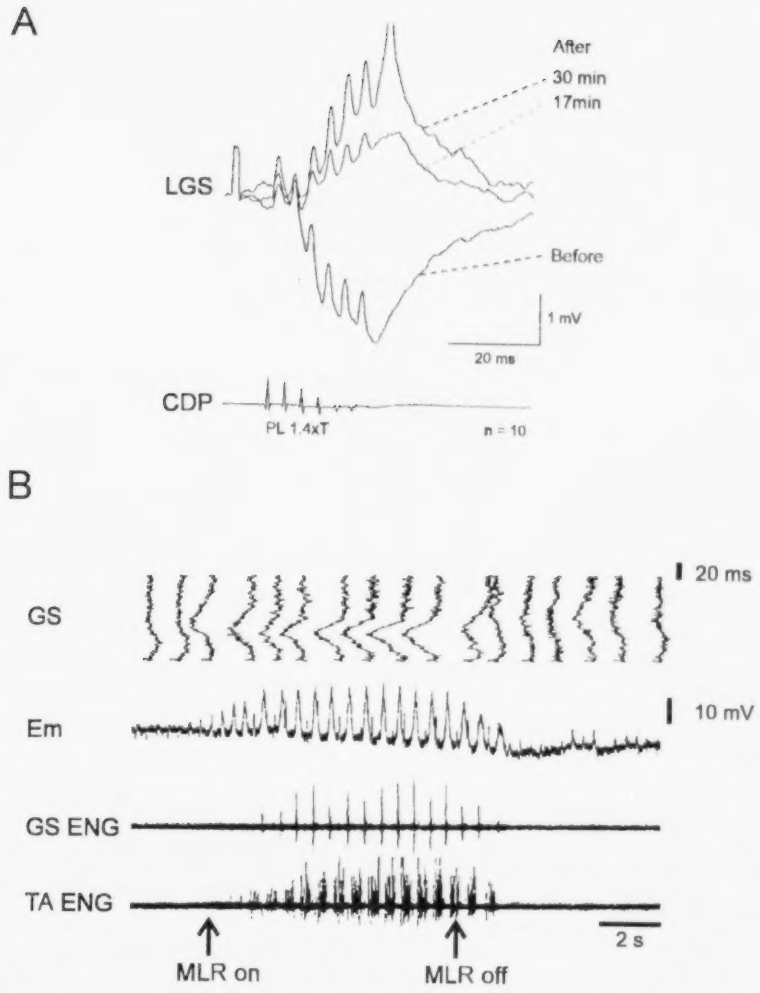

Figure 1-2: A. Excitatory actions of group I volleys recorded intracellularly in a LGS motoneuron after stimulating the PL nerve at 1.4 x T. Before the injection of L-DOPA and nialamide, stimulation of the PL nerve produced a small EPSP which was predominated by summating IPSPs which contribute to the classic non-reciprocal inhibition produced by the group Ib afferents. After treatment with L-DOPA and nialamide the IPSPs were inhibited and is replaced by a slow rising EPSP that increases progressively in size as the locomotor pathway opens. B. This trace shows the emergence of the group I excitatory response before and after stimulation of the MLR region in a paralyzed cat. A. From top to bottom: tilted vertically are the high gain intracellular responses (100 ms sweeps) to group I stimulation of the PL nerve (2 x T). Note that the first trace in this sequence shows the classic Ib inhibition, but the third trace in the sequence with MLR stimulation shows an excitatory response. The Em trace indicates the slow low gain intracellular record which indicates the locomotor drive potential. The last two traces are ENG traces from the triceps surae (GS) and the tibialis anterior (TA) respectively. (Modified from Gossard et al., 1994 with permission) *(PL plantaris)*

inhibitory to excitatory at the onset of locomotion (Conway et al. 1987; Pearson and Collins, 1993; Gossard et al., 1994; see figure 1-1). The pathway used to show this effect has been the one from plantaris (PL) to medial gastrocnemius (MG). This is because Pl does not project any group Ia afferents directly to the MG motoneurons (Eccles et al., 1957a), thus all effects onto MG from Pl are mediated by polysynaptic pathways. In the resting spinal cat, stimulation of the Pl nerve at group I strengths results in the classic inhibition of the extensor burst, originally described by Eccles (1957b) (figure 1-1). However, when the locomotor CPG is activated (by adding clonidine (an α-2 agonist)) similar stimulation of the Pl nerve during mid-stance results in an increase in the amplitude of the MG EMG (figure 1-1). It has been suggested that an oligosynaptic pathway that is only open during locomotion and accesses the extensor half-centre of the CPG is responsible for mediating this effect (Gossard et al., 1994). This access to the half-centre is supported by studies which show that electrical and mechanical activation of group I afferents can entrain and reset the locomotor rhythm (Conway et al., 1987; Pearson et al., 1992). Recent evidence shows that group Ia feedback from extensor muscles can also increase the amplitude of the extensor burst using the similar oligosynaptic pathways as group Ib afferents (Guertin et al., 1995a).

Intracellular studies have complemented the above findings and have recently found that more than one excitatory group I pathway is opened during locomotion. The first pathway has only been observed in fictively locomoting decerebrate cats (McCrea et al., 1995a). When extensor group I afferents are stimulated, a disynaptic EPSP can be observed in extensor motoneurons which is phasically modulated during the locomotor cycle (from now on this pathway will be referred to as the disynaptic pathway). It has been proposed (McCrea et al., 1995a; Guertin et al., 1995a) that this disynaptic pathway adds to the excitation produced by monosynaptic group Ia

pathways to enhance activity of the extensor burst. Evidence supporting this was obtained from the extensor electroneurogram (ENG) bursts which show a rise in the amplitude of the ENG at latencies consistent with activation of this disynaptic pathway (Guertin et al., 1995). The second pathway is opened after the administration of L-DOPA in spinal cats (figure 1-2A) or during MLR evoked locomotion in decerebrate cats (Gossard et al., 1994; see figure 1-2B) (from now on called the oligosynaptic pathway). Before L-DOPA is injected into an acute spinal cat, stimulation of group I extensor afferents [stimulus trains: 300 Hz (3-10 pulses)] causes summating IPSP's in the lateral gastrocnemius (LGS) motoneuron. When L-DOPA was added, the IPSP's were gradually reduced in size and replaced by long-latency EPSP's that, after 30 minutes, were large enough to cause a cell to fire an action potential. MLR stimulation was equally effective in opening up this long-latency excitatory pathway.

One of the questions that will be addressed in chapter 2 and 3 is whether stimulation of extensor group I afferents can increase the amplitude of the extensor burst in a walking animal. In all of the studies mentioned above there either has been no or a very reduced amount of afferent feedback. Prochazka et al.(1989) has estimated that feedback from muscle spindles from one extensor muscle can be in the order of 6 kilo impulses per second. When one considers the many other muscles in addition to the cutaneous and joint receptors, a considerable barrage of information would be absent from the paralyzed (or severely reduced) preparations above which may alter the expression of the studied reflex pathway.

Afferent control of the stance to swing transition

The previous section demonstrated that sensory input from extensor afferents can contribute to the amplitude of the extensor EMG. This section reviews the evidence that sensory feedback from the hindlimb can influence the timing of a cat's locomotor pattern. When a cat changes

speeds, adjustments are primarily made to the length of the extensor burst, while the flexor burst duration remains unaltered (Grillner, 1981; Halbertsma, 1984). One component of this adaptation is the peripheral afferent regulation of the transition from the stance to swing. At the present time there are two proposals regarding the afferent origin of this regulation. The first proposal is that a reduction in force feedback, due to the unloading of the extensor muscles at the end of stance, is the signal for initiating the transition from the stance to swing phase. This idea was advanced by Duysens and Pearson (1980) to explain the observation that stretches of the extensor muscles in decerebrate cats walking on a treadmill could inhibit the generation of the flexor bursts. The generation of the flexor burst was conditional on the force level being reduced below a level of 40 N. This led to the proposal that signals generated by the GTO and carried by Ib afferents could prolong the stance phase. The second proposal is that afferents from the hip signal the end of the stance phase. Grillner and Rossignol (1978) found that if a cat`s hindlimb was extended past 95 degrees, flexion was induced, but if the limb was kept in a flexed position, tonic extension occurred. It is quite likely a combination of afferents signaling both load and leg position forms the basis for the transition from stance to swing.

In the following sections the contributions from the extensor, flexor and cutaneous afferents to the transition from stance to swing will be described. Although it is possible that joint afferents may also contribute, these are not mentioned because of the relative lack of evidence implicating these afferents in the regulation of locomotion.

Extensor muscle afferents

Input from proprioceptors can reset or entrain the motor rhythm in many vertebrate and invertebrate species (Andersson and Grillner, 1981; 1983; Bässler, 1983; 1986; 1987; Clarac and Chrachri, 1986; Zill, 1985; McClellan and Jang, 1993; Pearson et al., 1992; Conway et al., 1987; Kiehn et al., 1992; for review see Pearson 1993).Accordingly, in the cat, input from group I

extensor afferents can reset and entrain (Conway et al., 1987; Pearson et al., 1992; Guertin et al., 1995a) the ongoing locomotor rhythm in clonidine and L-DOPA treated spinal cats as well as decerebrate cats. There is evidence suggesting that group Ib afferents that signal the load carried by the limb contribute substantially to the timing of the step cycle in clonidine and L-DOPA treated spinal cats. For example, vibration of extensor muscles (recruits group Ia afferents) did not entrain the rhythm while stimulation of the group I afferents or stretch of the actively contracting muscle did (Conway et al., 1987; Pearson et al., 1992). While Ib afferents can powerfully affect the timing of the locomotor rhythm, the evidence does not exclude the idea that group Ia afferents could contribute as well. Recent evidence demonstrates that vibration of the triceps surae, which preferentially recruits group Ia afferents, can powerfully reset the step cycle and increase the duration of the extensor ENG burst in fictively locomoting decerebrate cats (Guertin et al., 1995a). This has led to the proposal that group Ia and Ib afferents converge onto the oligosynaptic pathway open during locomotion (Angel et al., 1995; Guertin et al., 1995a).

In this thesis we look at the effects of stimulating the group I afferents in walking decerebrate (chapter 2) and intact cats (chapter 3). Chapter 2 demonstrates that stimulation of group I extensor afferents can result in profound changes in the stance duration of walking cats. Stimulation of the group I extensor afferents could powerfully extend the limb and delay the onset of swing. Chapter 3 extends the results to the intact walking animal and demonstrates that while stimulation of group I extensor afferents can affect the amplitude and duration of the extensor burst, these effects are dependent on the state of the animal.

Flexor afferents

Recently, work by Hiebert et al. (1996) has identified proprioceptive inputs from flexor muscles that can alter the timing of the step cycle in the hindlimb (cf Hiebert, 1996). To identify the afferents involved, Hiebert et al. (1996) stretched the iliopsoas (hip flexor) (IP), and/or tibialis

anterior (TA) (ankle flexor), and/or extensor digitorum longus muscles (EDL) during the stance phase in spontaneously walking decerebrate cats. Stretch of these flexor muscles inhibited the ongoing stance phase and promoted an earlier onset of flexion. One conclusion from this data is that activation of muscle receptors in both the hip and the ankle have similar effects on shortening the stance phase and initiating swing. Another conclusion is that the length sensitive spindle afferents from flexor muscles in the hip and ankle affect the transition from stance to swing, since vibration (EDL and/or IP) or electrical stimulation at group Ia strengths (EDL) or electrical stimulation at group II strengths (TA) each inhibit stance. In the high spinal L-DOPA treated preparation (Schomburg et al., 1993), stimulation of group II muscle afferents can reset the locomotor pattern to flexion a conclusion which supports the findings of Hiebert et al. (1996). However, a different conclusion was reached by Perreault et al. (1995). In this study, which used fictively locomoting decerebrate cats, stimulation of similar group II afferents from flexor muscles reset the locomotor rhythm to extension. It is conceivable that the use of different preparations (spontaneously walking (Hiebert et al., 1996) versus fictively locomoting decerebrate cats (Perreault et al., 1995) may explain why the results differed.

Cutaneous afferents

Nearly a century ago it was recognized that cutaneous stimulation of the distal foot could produce various excitatory extensor reflexes in animals at rest (Extensor thrust: Sherrington, 1906, positive supporting reaction: Magnus, 1926, toe extensor reflex: Engberg, 1964), and hence it was hypothesized that cutaneous reflexes might serve to reinforce the stance phase during locomotion. At the same time, Sherrington realized that cutaneous input from the footpad alone was not essential to walking in the intact animal, "- in the intact animal (cat, dog), severance of all the nerve trunks directly distributed to all four of the feet up to and above the wrists and ankles impairs walking so little as to make it highly unlikely that the loss of

receptivity of the feet destroys any large reflex basis of these acts" (Sherrington, 1910). Since that time it has been shown that the extent to which cutaneous input affects the step cycle depends on the phase of the step cycle the perturbation is delivered to and on the strength and type of stimulation (Duysens and Stein, 1978; Duysens, 1977; Duysens and Pearson, 1976; Grillner and Rossignol, 1978; Forssberg, 1979). Activation of cutaneous afferents can elicit complex patterns of muscle activity. For example, stimulation of cutaneous afferents from the dorsum of the foot can evoke the well known stumbling corrective response (Forssberg, 1979). This response ensures an exaggerated flexion response occurs if a perturbation is encountered during the swing phase, while in contrast if the perturbation is encountered during stance the extensor burst is enhanced. However, the role of cutaneous afferents in the normal control of the stance to swing transition is unclear.

When discussing this issue it must be kept in mind that cutaneous afferents can, in theory, signal length, pressure (force), and velocity information during walking as well as nociception, depending on the type of receptor and its placement (Loeb, 1981). Stimulation of the sural and posterior tibial nerve can increase the amplitude and the duration of the extensor burst (Duysens, 1977; Duysens and Pearson, 1976; Duysens and Stein, 1978; Guertin et al., 1995a). For example, weak electrical stimulation of the sural nerve during late flexion terminates flexion prematurely and resets the rhythm to extension. In contrast, if the same stimulus is applied during early flexion, the flexor bursts are frequently augmented and the subsequent extensor burst is shortened (Duysens 1977). In many cases, stimulation of the sural nerve during late stance increases the duration and the amplitude of the extensor burst by over 100% and completely abolishes the ongoing ipsilateral flexor burst (Duysens and Pearson, 1976, c.f. Labella and McCrea, 1990). It is not known whether the spinal circuitry mediating this effect shares common interneurons with proprioceptive afferents from the extensor and flexor muscles.

Plasticity of reflex pathways

The previous section has described short term changes in reflex pathways (such as reflex reversals) evolving over a cycle of movement. More persistent changes in reflex efficacy are often observed in neurophysiology, including the remapping of the auditory system in the barn owl (Knudsen and Knudsen, 1990), the adaptation of the vestibulo-occular reflex (Lisberger, 1988), the ability of chronic spinal cats to regain their ability to walk following regular training (Barbeau and Rossignol 1994; 1987; Barbeau et al., 1993; Edgerton et al., 1992; Lovely et al., 1986) and the remapping of the spinal cord sensory map after injury (Devor and Wall, 1978). However, relatively little is known about how reflex pathways that control locomotion adapt in response to new environmental conditions or injury.

Behavioral studies

Yanagihara et al. (1993) found that chronic decerebrate cats can adapt their interlimb coordination appropriately when their limbs are driven at different velocities relative to each other. Animals were mounted over a treadmill, which allowed the left forelimb of the animals to be driven at different velocities. When the left forelimb was driven at twice the velocity of the other three limbs, there was an immediate disruption of the stance phase of the left forelimb. However, within 50 steps the step cycle of the left (and right) forelimb stabilized and subsequent perturbations showed an immediate adaptation indicating that the decerebrate animal had some 'memory' of the perturbation . Lou and Bloedel (1988) have shown that the trajectory of a forelimb in decerebrate locomoting ferrets can be conditioned to avoid an obstacle. What is quite surprising (figure 1-3A) is that the new behavior is learned within 5-15 trials, and moreover the behavior can be eliminated after a number of trials if the bar is removed. The authors later combined recordings from Purkinje cells (figure 1-3B) and monitored both simple and complex spikes during perturbed and unperturbed walking (Lou and Bloedel 1992). During unperturbed

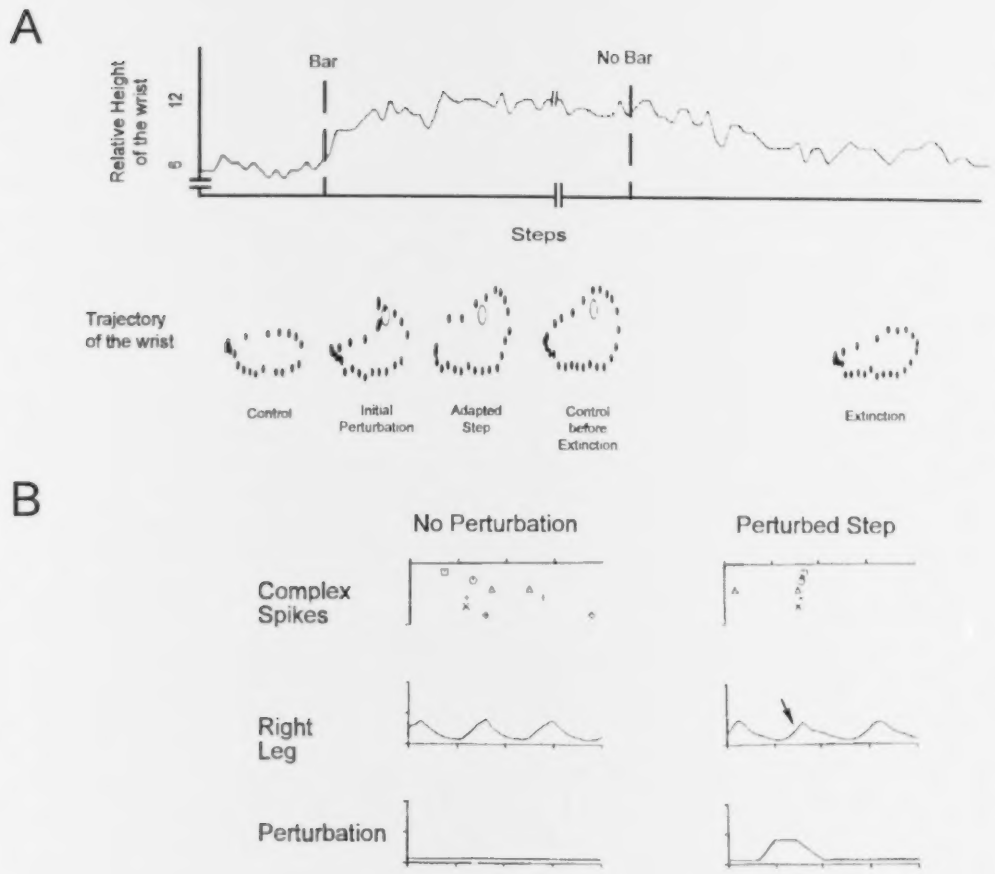

Figure 1-3: A figure showing the ability of a decerebrate ferret to adjust the trajectory of its forelimb in response to a mechanical perturbation. A. The top trace refers to the relative height of the wrist during normal locomotion and perturbed locomotion (between the two vertical lines). Note that the ferret learned to lift its wrist and avoid the obstacle within a short number of trials (<6) after a mechanical bar was introduced. The bottom traces show the typical trajectories of the limb in control, immediately after perturbation, after adaptation, and the extinction of the stimulus after the removal of the bar. (Redrawn from Lou and Bloedel, 1988 with permission). B. This figure shows results from another experiment in which recording from Purkinje cells were obtained from the decerebrate walking ferret in control situations and after the introduction of the mechanical bar. Note that in the control trace the complex spikes were not correlated to any particular point in the step cycle of the ferret, however after introduction of the bar the complex spikes became correlated to the perturbation. Each point on the graph refers to the recordings of complex spike activity from an individual Purkinje cell (reproduced from Lou and Bloedel, 1992 with permission)

walking the complex spike activity was not correlated with the step cycle. When the bar was introduced, the production of the complex spikes became highly correlated to the perturbation. Studies by Yanagihara and Udo (1994) confirmed that in the decerebrate locomoting cat similar perturbations of the forelimb lead to synchronization of the complex spikes (c.f. Matsukawa and Udo, 1985) and to an altered firing of the left and right Deiter's nucleus (Udo et al., 1982). Bloedel (1992) has hypothesized that synchronization of climbing fibre input activates sagtitally aligned Purkinje cells and causes an on-line change in the efficacy of mossy fibre inputs. This would lead to an alteration in the output of cerebellar nuclear neurons every time the perturbation is encountered by the animal. Consistent with this idea, preliminary evidence suggests that ablation of areas of the cerebellum does not abolish the acquisition of the bar avoidance task in decerebrate ferrets (Bloedel et al., 1991), although it does alter the performance of the movement. It is not known whether the site of plasticity for all the behaviors discussed above is located in the brainstem or cervical spinal cord. It is possible that the site of plasticity may not be located within a defined area and may be distributed across the spinal cord and brainstem (Bloedel 1995; Dr. James Bloedel -- personal communication).

While the adaptation described above occurs quite rapidly, a slower type occurs when descending or sensory information is eliminated in intact cats. For example, immediately after a unilateral deafferentation, such cats can not walk, but yet after as few as two weeks these animals regain the ability to bear weight on the affected limb and place it accurately during locomotion (Goldberger, 1977; cf Wetzel et al., 1976). There are permanent deficits in the kinematic pattern of the affected limb. For example, while the animal can use the affected limb to walk quadrupedally, it can not use it to walk bipedally, choosing instead to hop while dragging the affected limb (Goldberger, 1988a). Also, it can no longer walk in challenging environments such as small beams. It is not known what mechanisms mediate this type of recovery although

supraspinal inputs do contribute since if the spinal cord is transected after recovery of function, the animal loses the ability to use the affected limb and no further adaptation is possible (Goldberger, 1977). If a partial deafferentation is performed, the recovery of locomotion is much better, for example they can now bipedally walk and negotiate small beams. Interestingly, if a hemisection is made in these animals they do recover suggesting that increased use of sensory input mediates the recovery in partially deafferented animals (Goldberger, 1988b). It is clear from the these studies that a remarkable recovery of function is possible after injury of the CNS. Supraspinal pathways may mediate part of this adaptation by substituting for absent reflex mechanisms only when no other sensory input is available (Goldberger, 1977; Goldberger, 1988b).

One of the missing links in the behavioral research is that while they demonstrate that dramatic plasticity can occur after injury, there has been no analysis of which reflex pathways are affected. This is mainly due to the lack of knowledge at the time these studies were undertaken of which reflex pathways could affect locomotion.

Spinal Pathways

As the previous section has revealed, the circuitry of the spinal cord is plastic and can adapt to changes in input from descending and peripheral inputs (Mendell, 1984). Spinal cord injury in humans causes changes in the strength of monosynaptic and polysynaptic reflexes to occur (Mendell, 1984). Adaptation of the spinal cord circuitry not only occurs after transection of the cord but also in response to injuries of the peripheral nerves and dorsal roots. The reflex pathway that has been predominantly used to assess the effects of injuries to the peripheral nervous system has been the monosynaptic reflex from group Ia afferents onto α-motoneurons. Eccles and colleagues were among the first to show that cutting a peripheral nerve results in diminution of the Ia EPSP (Eccles et al, 1959; cf. Eccles and McIntyre, 1953). This decrease occurs after only

7-10 days of the cut and within three weeks the magnitude of the EPSP has been reduced to 50% of control values (Eccles et al., 1959; Gallego et al., 1979). This decrease is not due to death of the sensory neurons since if the sensory axon reinnervates the muscle the EPSPs return to normal (Goldring et al., 1980). A study by Webb and Cope (1992) showed that after the action potentials arising from MG nerves were chronically deactivated by TTX, the amplitude of the monosynaptic EPSPs were increased. This suggests that axotomy and not disuse is the causal factor in the loss of synaptic efficacy after nerve section. When extensor muscles are denervated and the remaining muscles are forced to take the increased load, the synaptic efficacy of the intact afferents increases (Eccles et al, 1962). This increase occurs in less than 5 weeks and shows that adaptive compensation is possible after muscle afferents are denervated.

In chapter 4 and 5 we describe the plasticity that occurs in the extensor group I pathway following axotomy of the LGS nerve. These studies provide a bridge between the behavioral studies and those which have concentrated on plasticity of spinal reflex pathways. This is because stimulation of group I extensor afferents has an observable effect on the stepping behavior of an animal. In chapters 4 and 5, we describe that the effects of stimulating the group I extensor afferents from the axotomized and synergistic nerves change after less than one week. The time course for the recovery closely parallels the emergence of these plastic effects, suggesting that they may be associated.

Summary

To summarize, this thesis will explore whether stimulation of group I extensor afferents can control of the stance to swing transition. One of the main themes of this thesis is exploring the functional importance of extensor group I stimulation in walking cats and especially the normal cat.

It is important to outline the functional role of reflex pathways from proprioceptors as their role has been a matter of controversy in the past. Thus, this thesis helps in our understanding of how proprioceptive reflex systems may contribute to normal stepping.

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Chapter 2

Stimulation of the group I extensor afferents prolongs the stance phase in walking cats[2]

Introduction.

Sensory feedback from the hindlimb can influence aspects of a cat's locomotor pattern during walking (for review see Rossignol et al., 1988). For example, spinal and decerebrate cats can adapt their stepping to different treadmill speeds (Forssberg and Grillner 1973; Forssberg et al. 1980). When a cat speeds up or slows down, adjustments are made to the length of the extensor burst, while the flexor burst remains unaltered (see Grillner 1981 for review). This adaptation is caused by afferent signals that regulate the transition from the stance to the swing phase.

Presently, it is not known which group(s) of afferents regulate the stance to swing transition. However, two hypotheses have been proposed. The first is that hip afferents signal the end of the stance phase when the hip angle extends past a critical angle (Grillner and Rossignol 1978; Andersson & Grillner 1978,1981). Grillner and Rossignol (1978) found that if a cat's hindlimb was held at a hip angle less than 95 degrees, stepping could be stopped in that limb. When the hip was extended past 95 degrees, flexion resumed. The second hypothesis is that a reduction in force feedback, due to unloading of the extensor muscles at the end of stance, is the

[2] A version of this chapter was published. Whelan, P. J., Hiebert, G. W., and Pearson, K. G. Stimulation of the group I extensor afferents prolongs the stance phase in walking cats. Exp. Brain Res. 103:20-30, 1995. As the primary author of this chapter, I was heavily involved in the performance and execution of each experiment, and I performed all of the subsequent data analysis. This chapter incorporates numerous suggestions from Dr. K.G. Pearson regarding its style and content.

signal for initiating the transition from the stance to swing phase. This idea was advanced by Duysens and Pearson (1980) to explain the observation that stretches of the extensor muscles in decerebrate cats walking on a treadmill could inhibit the generation of flexor bursts. The generation of the flexor burst was conditional on the force level being reduced below 40N and was not dependent on the length of the muscle. This led to the proposal that signals generated by golgi tendon organs (GTO) and carried by Ib afferents could prolong the stance phase. Since then, evidence supporting this proposal has come from numerous studies on reduced L-DOPA or clonidine treated spinal preparations: 1) electrical stimulation of the group I afferents can inhibit the generation of flexor bursts during fictive locomotion (Conway et al. 1987), 2) extensor Ib afferents can excite the extensor motoneuronal pool during locomotor activity (Pearson and Collins 1993), 3) the locomotor rhythm can be reset and entrained by input from extensor group Ib afferents (Conway et al. 1987; Pearson et al. 1992), and 4) vibration, which preferentially recruits Ia afferents cannot reset or entrain the rhythm (Conway et al. 1987; Pearson et al. 1992).

The primary objective of this study was to establish whether stimulation of group I afferents in extensor nerves of hindlimbs can alter the magnitude and timing of extensor burst activity in the spontaneously walking decerebrate cat. The specific nerves chosen for stimulation were selected from ankle and knee extensor muscles because: 1) it allowed us to attribute any changes in the magnitude or duration of the extensor burst to activation of the low-threshold (group I) afferents, and, 2) we could test the hypothesis formed from previous studies in immobilised preparations that posited a functional role for group I afferents in the timing of the transition from stance to swing during normal walking (Gossard et al. 1994; Pearson et al. 1992).

Methods

All animals used in this study were cared for in accordance with the guidelines published by the American Physiological Society. Experiments were carried out in ten adult cats (2.5 to 4.0 kg) decerebrated at the premammillary level. Two animals failed to walk regularly and did not provide useful data.

All animals were initially anesthetized with halothane and the trachea was cannulated. The right carotid artery was ligated and the left cannulated to monitor blood pressure. The left jugular vein was cannulated to allow administration of fluids and drugs. Following this procedure, the nerves supplying the plantaris (Pl), lateral gastrocnemius-soleus (LGS), and the vastus lateralis and vastus intermediate (VL/VI) muscles were exposed and transected close to the muscle. A 1 cm length of each nerve was freed and tied into a small bipolar stimulating cuff electrode (see figure 2-0). The cuffs containing the electrodes were about 6mm long with an inside diameter of about 3mm. The bared ends of two teflon coated stainless steel wires (Cooner Wire Company, AS631) were looped within the cuffs and acted as the stimulating electrodes. The wires as they exited the nerve were arranged so that movement of the cuff in relation to the nerve was reduced during walking.

A laminectomy was performed at the L4 level and bipolar recording electrodes (Cooner Wire Company, AS632) were then placed on the dorsum of the spinal cord with one electrode placed below the L4 spinal vertebrae and the other below the L5 spinal vertebrae. The strength of the stimulus to any nerve was measured as a multiple of the smallest observable cord dorsum potential.

Bipolar stainless steel recording electrodes (Cooner Wire Company, AS632) were sewn into the following hind leg muscles to record electromyographic (EMG) activity during walking:

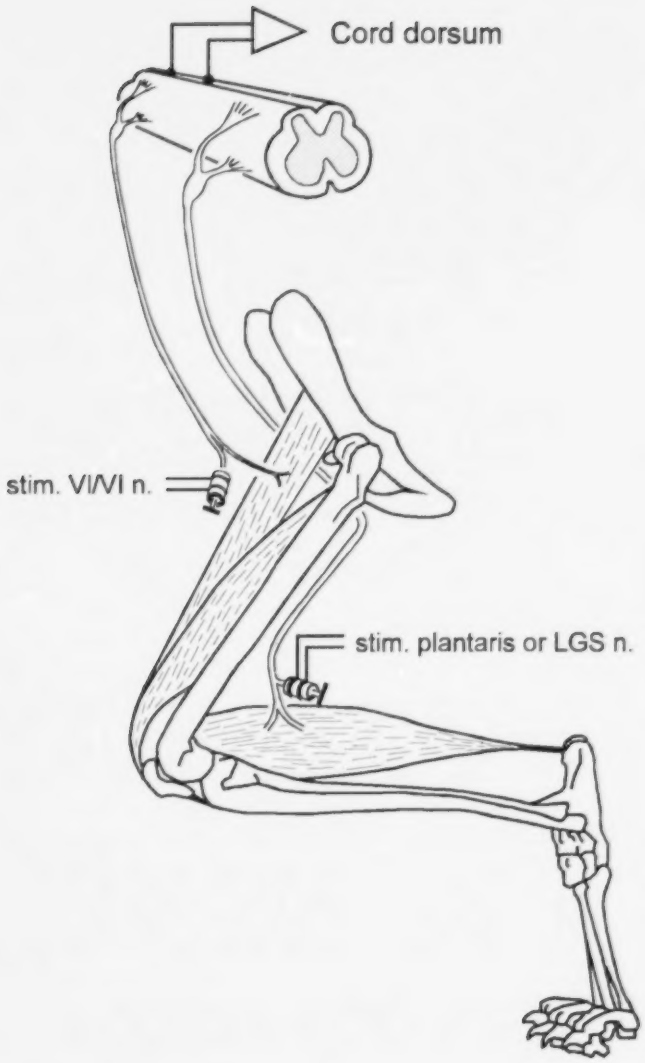


Figure 2-0: Stimulation of the extensor nerves was accomplished by transecting the vastus lateralis and vastus intermedius (VL/VI), lateral gastrocnemius (LGS) and plantaris nerve close to their parent muscles and implanting a latex cuff around the proximal end of each nerve. The strength of the stimulus to any nerve was measured as a multiple of the smallest observable cord dorsum potential (figure courtesy of K. Pearson).

Left leg - medial gastrocnemius (extensor of the knee) (MG), vastus medialis (extensor of the knee) (VM), semitendinosus (hip extensor and knee flexor) (St) and iliopsoas (hip flexor) (IP) (implanted in three cats); right leg - vastus lateralis (VL) and semitendinosus. The wires from both the stimulating and the EMG electrodes were led subcutaneously to a multipolar connector near the middle of the animal's back.

Following placement of the stimulating and EMG recording electrodes the animal was placed in a stereotaxic head holder and positioned above a motor-driven treadmill. The body was supported by a sling under the abdomen. The animal was then decerebrated by transecting the brainstem at a 50° angle from the anterior edge of the superior colliculus using a sterotaxically guided spatula. The anesthetic was discontinued after decerebration.

In 6 of the animals, locomotion began to occur spontaneously about 1 hour after removal from the anesthetic. The speed of the treadmill was set at between .25 and .30 m/s depending on the animals stepping pattern. Spontaneous bouts (each lasting 1 to 2 minutes) of locomotion occurred intermittently for 30 minutes to 4 hours. In two animals that did not walk spontaneously, and in the remaining animals following cessation of spontaneous bouts of walking, an attempt was made to induce locomotion by electrical stimulation of the mesencephalic locomotor region ((Horsley-Clarke co-ordinates [P2,L4,H6]), Shik and Orlovsky 1966). The stimulus parameters were a continuous train at 15Hz of 100μA, 1ms pulses.

During periods of locomotor activity, the cuffed nerves were stimulated either individually or in combination. Usually a stimulus train was triggered at a preset delay following the onset of burst activity in the left MG muscle. The onset of MG burst activity was detected by the on-line monitoring of the rectified and filtered EMG by an interactive program on a Zenith 386 computer using a Transduction DT2821 analog to digital interface board. Additionally, in 3 cats stimulus

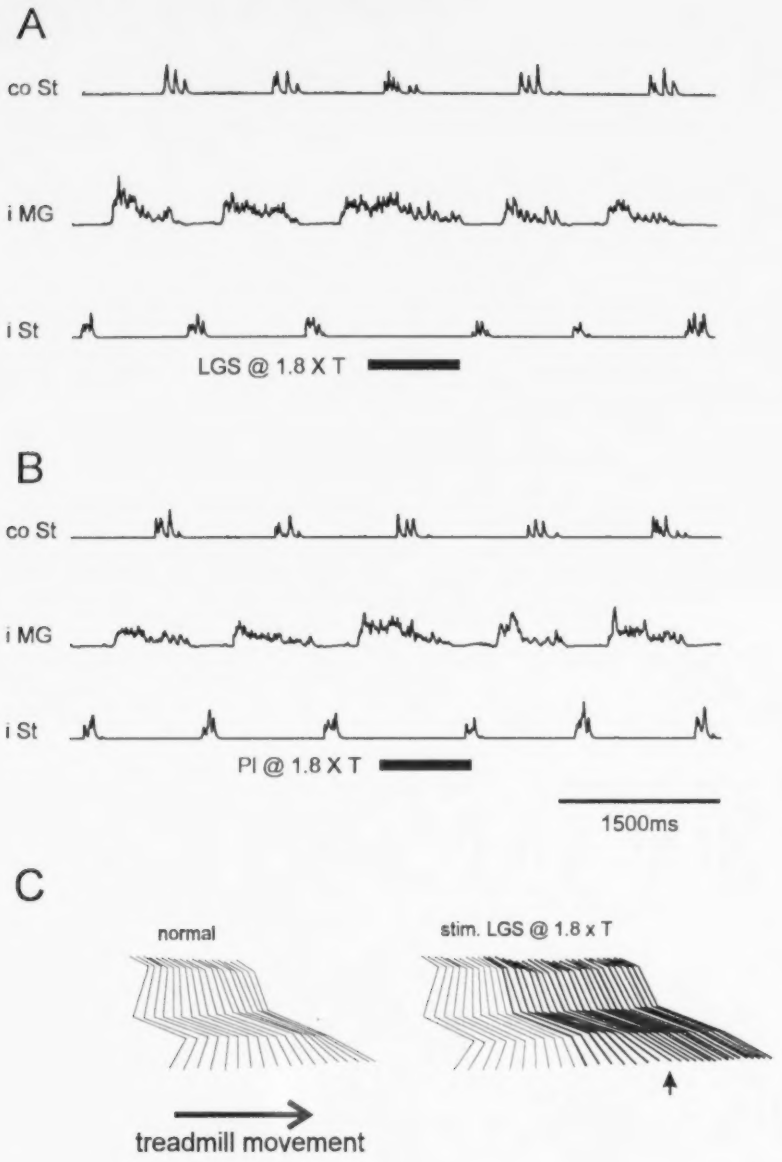


Figure 2-1: Stimulation of group I afferents can prolong the stance phase and delay the flexor burst. Both sets of records in A) and B) show rectified and filtered EMG responses from the muscles of the hindlimb during walking. A) and B) Effects of stimulating the LGS and Pl nerves at 1.8xT respectively (stimulus trains: 800ms duration, 150Hz, 200ms delay following the start of the MG EMG burst). The stimulus trains were monitored for stability by recording the cord dorsum response. Note that in A) the duration of the ipsilateral MG (i MG) burst was prolonged (middle trace for both graphs) and in A) and B) the ipsilateral St flexor burst (i St) was delayed. However, LGS stimulation usually delayed the flexor burst for the duration of the stimulus, whereas plantaris stimulation was typically less effective. The contralateral St (co St) showed only a modest increase during stimulation in both cases (top trace in each plot). C) Stick figures graphically comparing the normal stance phase (left trace) with the perturbed stance phase (right trace). The time of stimulus onset is indicated by the black lines and the small arrow. Note the maintained extension of the ankle joint that occurred during the stimulus train. The large black arrow indicates the direction of the movement of the treadmill belt.

trains were triggered off the Iliopsoas (Ip) flexor burst to establish whether the flexor burst was inhibited during extensor nerve stimulation.

All data were recorded using a Vetter 4000A PCM recorder. Later, selected sequences were stored on computer disc using the Axotape (Axon Instruments) data acquisition system installed on a Microexpress 486 computer. Data analyses were carried out using custom programs that could retrieve data from the axotape files. In 3 cats that walked for long periods of time, a statistical analysis of the data was possible. The cycle periods before, during, and after the stimulus were calculated for sequences of walking. The mean and standard deviation were calculated for these cycle periods and Student's t-tests were administered to detect significant differences between the conditions. All analyses were performed at the 95% confidence level.

Six experiments were recorded using high speed cinematography. Kinematic data, including stick figures, were obtained by analyzing selected video sequences using Video Blaster software (Creative Labs Inc.) and custom designed programs.

Results

Influence of the group I afferents on the cycle period

Previous studies in spinal animals have found that stimulation of the group I afferents in the nerves supplying the quadriceps, triceps surae and plantaris muscles can reset the locomotor rhythm (Conway et al 1987; Gossard et al., 1994; Pearson et al. 1992; Pearson and Collins 1993). The characteristic feature of this resetting effect is that extensor bursts are prolonged when the stimulus is delivered near the end of the extensor phase. One conclusion of the present investigation is that stimulation of these nerves in walking decerebrate cats prolongs the extensor phase of the step cycle. This is illustrated in figure 2-1 in which the LGS and PI nerves were stimulated at 1.8 times threshold (xT) for 800ms beginning about 200ms after the onset of burst

activity in the MG muscle. In both cases the duration of the extensor phase was prolonged. The LGS nerve stimulation consistently delayed the onset of flexor burst activity until the stimulus train ended. Generally, stimulation of either the Pl nerve or the VL/VI nerves did not always prevent the onset of flexor burst activity, whereas stimulation of the LGS nerve did. Data for stimulation of the three extensor nerves in a single animal is shown more quantitatively in figure 2-2.

In one cat where a stimulus train of 800ms delayed the onset of flexor activity until the end of the stimulus, we lengthened the stimulus train to establish whether the locomotor rhythm could be suppressed for long periods. This only occurred for stimulation of the LGS nerve. Figure 2-3 shows that stimulus trains (1.8xT) of up to two seconds completely suppressed the locomotor rhythm in the ipsilateral leg, whereas the stepping in the contralateral leg continued at the same rate. Figure 2-3B shows the quantification of the above results for three stimulus train lengths. The prolongation of extension was obvious to visual inspection. During the stimulus train the leg was held in extension at the ankle, knee, and hip. The extension at joints other than the ankle suggested that stimulation of group I afferents in LGS led to the maintenance of activity in all the extensor muscles that are normally active during the stance phase. An example of this can be observed in figure 2-8D, during which stimulation of the LGS nerve produced an excitation of the VL muscle.

In reduced preparations, the fictive step cycle can be completely reset by group I afferent stimulation (Conway et al. 1987, Pearson et al. 1992). In these cases the contralateral pattern becomes phase-locked to the ipsilateral rhythm. In our experiments the timing of the contralateral limb was affected by stimulation of the ipsilateral extensor group I afferents, however the step cycle was not usually reset. In figure 2-1 for example the contralateral flexor

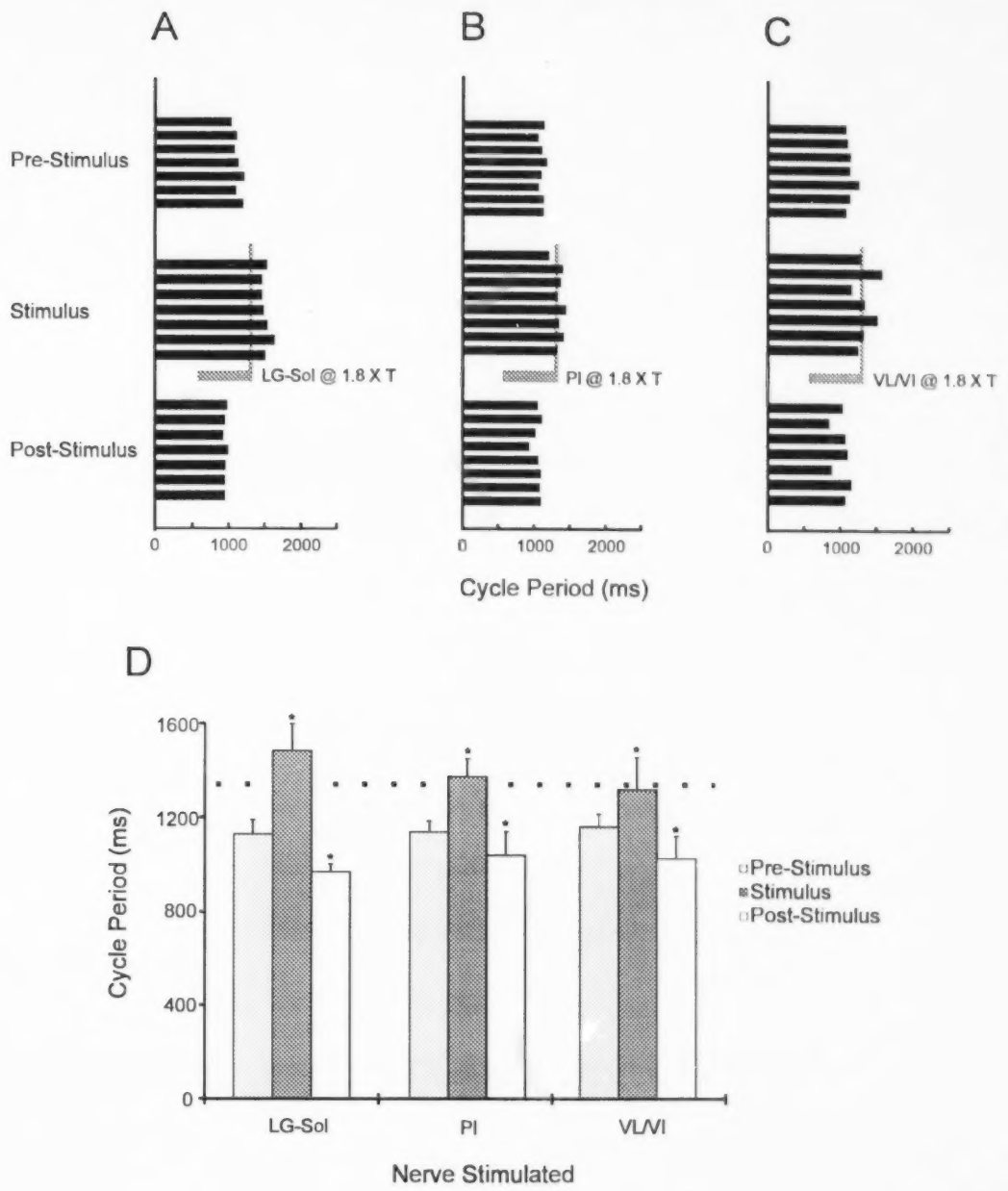


Figure 2-2: Bar graphs showing the cycle period (measured from successive St flexor bursts) for step cycles before, during, and after extensor nerve stimulation. A) B) and C) illustrate the results from stimulation of the VL/VI (n=7), PI (n=8) and LGS (n=7) nerves at 1.8xT respectively. The grey bar indicates the time that the stimulus was on during the stimulated step cycle. In each graph a single step cycle is represented by a black bar. The initiation of the flexor burst was delayed by stimulation of each of the nerves. Note that the LGS nerve delayed the flexor burst past the end of the stimulus train, whereas the PI and VL/VI nerves typically had a less powerful effect. D) This graph illustrates the averaged responses from A) B) and C). The dotted line indicates the time of stimulus offset (standard deviation bars are shown for each trial; * = statistically different from control steps ($p<.05$))

burst was delayed accompanying the delay in the ipsilateral flexor burst. This strong inhibition between the flexor bursts on the ipsilateral and contralateral legs ensured that no overlap occurred during stepping. When the extensor group I stimulus extended the duration of the ipsilateral extensor burst for more than one step cycle the ipsilateral flexor burst was initiated 180 degrees out of phase with the contralateral flexor burst.

The strong influence of stimulating group I afferents in the LGS nerve on the locomotor cycle was not observed in an earlier investigation by Duysens and Stein (1978). The most obvious difference between that study and our own were the parameters of the stimulus train. Trains of stimuli at 60Hz lasting for about 100ms where used in the study by Duysens and Stein, and these trains were delivered at a constant rate throughout the step cycle. When these stimulus parameters were applied in our study we also failed to observe any significant influence ($p>.20$) on the locomotor cycle. The reason for the absence of an effect was that the stimuli frequency was too low. Figure 2-5 shows that the cycle period was significantly increased ($p<.05$) when the LGS nerve was stimulated with 150Hz trains triggered after the onset of MG burst activity. The minimum frequency needed to consistently prolong the cycle period was about 100Hz.

The strength of the stimulus required to produce a significant lengthening of the cycle period was between 1.3 and 1.6xT. The examples shown in figure 2-6 are for the LGS nerve, but similar data were obtained for the Pl and VL/VI nerves. No noticeable effect on the duration of the stance phase could be observed with stimulus strengths of 1.3xT and less. Since a stimulus at 1.3xT is sufficient to activate a large fraction of the group Ia afferents (Jack, 1978; Coppin et al., 1970), these afferents in isolation are probably not able to influence the locomotor rhythm generator. A similar conclusion has been reached from studies in reduced spinal preparations (Conway et al. 1987; Pearson et al. 1992; Pearson and Collins

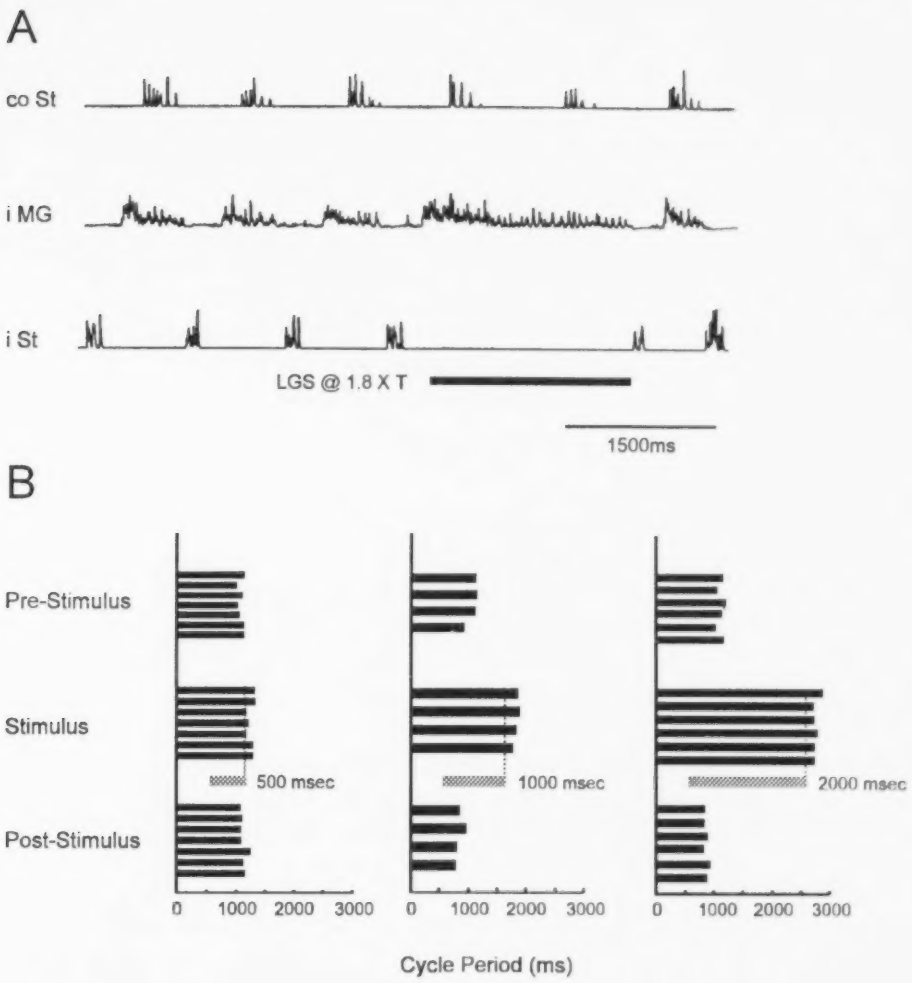


Figure 2-3: Increasing the duration of the LGS stimulus can delay the flexor burst for extended periods. A) Rectified and filtered EMG responses from a single animal during stimulation of the LGS nerve at 1.8xT (stimulus trains: 2000ms duration, 150 Hz, 200ms delay). Note that the MG burst was prolonged and the St burst was delayed until termination of the stimulus train. Also note that the contralateral stepping pattern was not altered by the stimulation. B) The three bar charts illustrate the effect of increasing the duration of the LGS stimulus pulse from 500ms (left graph) to 1000ms (middle graph) to 2000ms (right graph). Each graph shows a step cycle immediately before the stimulus, during the stimulus, and immediately after the stimulus. Note that in all cases the LGS stimulation held off flexion for the duration of the stimulus train (indicated by the grey bar).

1993), although a recent abstract suggests that stimulation of group Ia afferents (1.15xT) in the Pl, MG or LGS nerves can produce an increase in the ampitude of the extensor burst in fictive MLR preparations (Guertin et al., 1993).

Simultaneous stimulation of two nerves also had no significant effect when the strengths were below 1.3xT. However, at higher strengths we observed spatial summation when two nerves were stimulated simultaneously (figure 2-7). The examination of spatial summation at 1.8xT was only possible when the individual stimulus trains did not completely suppress the generation of flexor bursts. Thus, we were usually restricted to examining the effects of simultaneously stimulating the Pl and VL/VI nerves, since LGS nerve stimulation usually maintained extensor activity for the duration of the stimulus. In 2 of 3 animals we observed that simultaneous stimulation of the Pl and VL/VI nerves produced a greater delay in the flexor burst onset than stimulation of either one of these nerves alone. Moreover, during many sequences there was a nonlinear summation of effects since the combined effect was more than the sum of the individual effects. In two animals we reduced the stimulus strength to each of the Pl (1.4xT) and the LGS (1.3xT) nerves to sub-threshold levels for producing an effect on the cycle period. Although neither produced an effect when they were stimulated alone, simultaneous stimulation produced a significant increase in the cycle period (not shown).

Influence of the group I afferents on extensor burst amplitude

In the acute spinal cat stimulation of extensor Ib afferents in quiescent preparations results in inhibition of the extensor EMG burst (Pearson and Collins 1993). However, it has been recently established that stimulation of extensor group Ib afferents can increase the magnitude of extensor bursts during the extensor phase in clonidine treated spinal cats (Pearson and Collins,

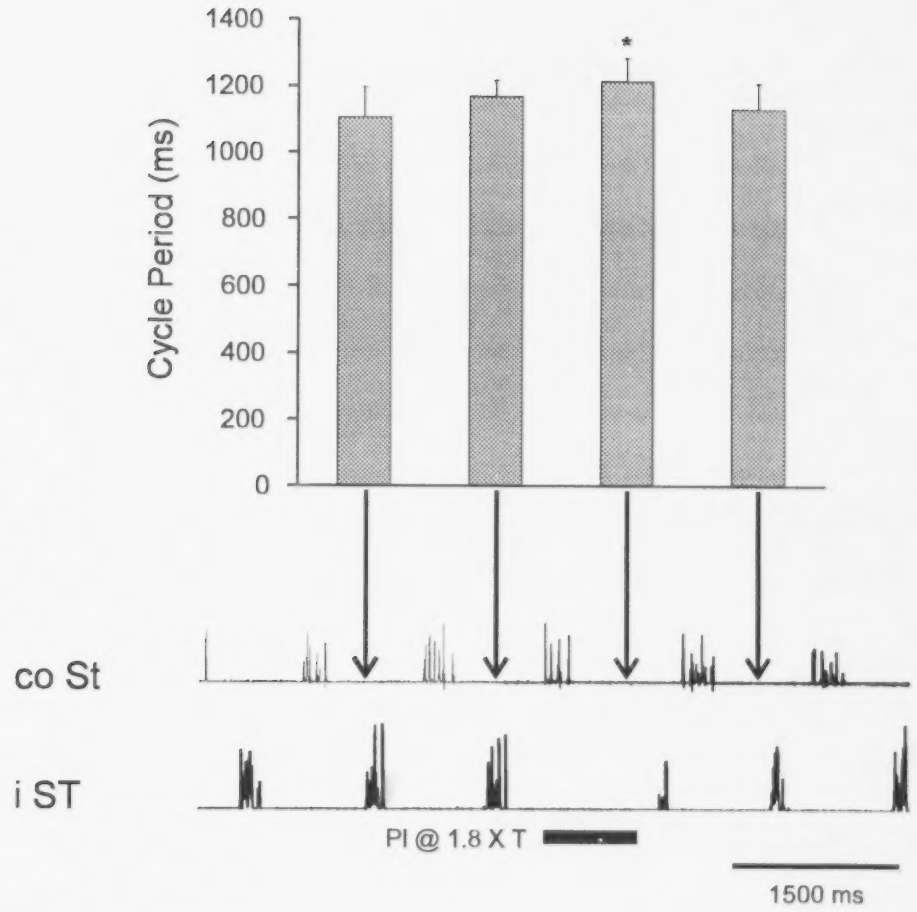


Figure 2-4: The duration of the contralateral step cycle showed a modest increase during stimulation of the ipsilateral hind limb. The rectified and filtered EMG traces on the bottom illustrate the effect on the cycle period of the ipsilateral and contralateral St following stimulation of ipsilateral Pl nerve (trains: 800ms duration, 1.8xT, 150 Hz, 200ms delay). The bar graph represents the average (n=6) cycle period for the contralateral St during each period indicated on the sample EMG plot (standard deviation bars are indicated for each trial; * = statistically different from control steps ($p<.05$)).

1993) indicating that positive feedback from Ib afferents may occur during walking. We asked whether these effects could be observed in a walking animal. The pathway that we examined was from Pl to MG, and was chosen because: 1) It allowed a comparison between walking and immobilised preparations and, 2) the pathway contains only a few monosynaptic connections from the group Ia afferents (Eccles et al. 1957a), which allows the effects to be attributed to polysynaptic projections from the group I afferent system. The results show that the effects from stimulation of Pl on the amplitude of the MG burst were variable. Figures 2-8A and 2-8B illustrate examples from an animal that consistently showed an increase in the MG EMG magnitude that occurred 60-70ms after the stimulus onset (Pl @ 1.8xT). The excitatory effect typically was pronounced during the falling phase of the extensor burst. In 2 animals, no excitatory effect occurred on stimulation of the plantaris nerve (figure 2-8C) even though an increase in cycle duration was observed. Two animals showed an excitatory response that was variable but on average showed a small increase during the falling phase of the excitatory burst. One animal showed no increase in the cycle period or amplitude upon stimulation of the Pl nerve.

Since group I afferents from quadriceps muscles make only weak monosynaptic connections to MG motoneurones, and the ankle extensors also make weak monosynaptic connections onto the quadriceps muscles (Edgley et al. 1986), it was possible to study the effects of group I stimulation on EMG burst amplitude from: 1) LGS onto the VM burst, 2) Pl onto the VM (or VL in one animal) burst, and 3) VL/VI onto the MG burst. Stimulation of the ankle or knee extensor nerves at group I strength produced a generalised excitation of the extensor musculature. Figure 2-8D shows the late excitation (~200ms) that developed in the VL muscle upon stimulation of the LGS nerve. When the Pl nerve was stimulated the VL EMG showed an excitatory response at

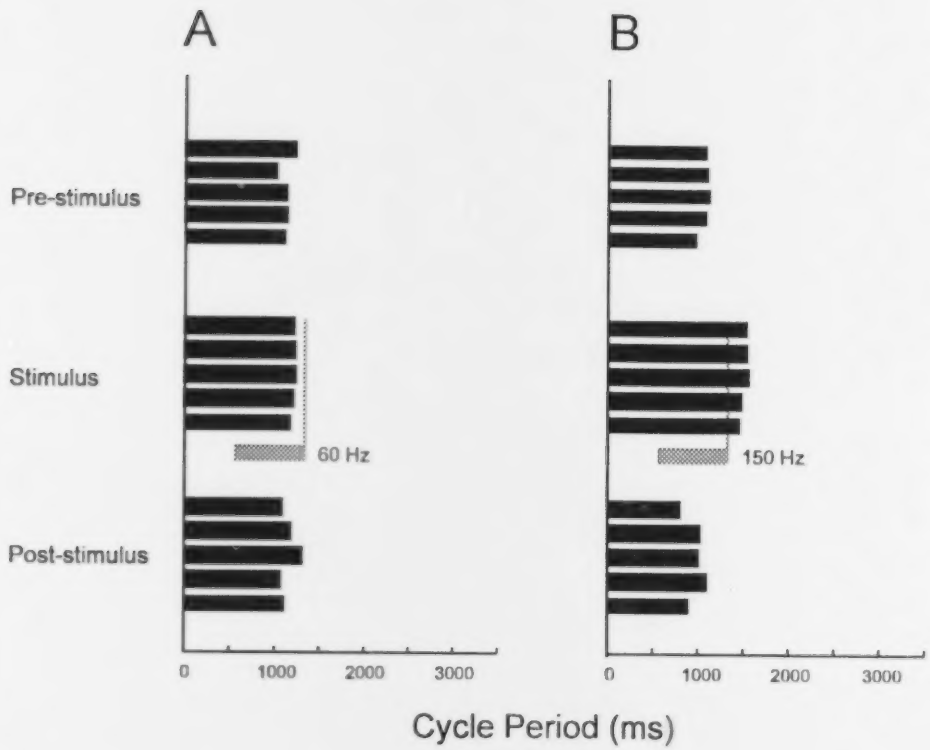


Figure 2-5: The increase in the cycle period was dependent on the frequency of the stimulus train. Both graphs illustrate 5 step cycles from the same animal immediately before, during and immediately after a stimulus train was delivered to the LGS nerve (train: duration 800ms, 1.8xT, 200ms delay). The grey line indicates the duration of the stimulus train during the stimulated step cycle. Note that when the frequency of the stimulus was set at 60 Hz (A) there was no appreciable effect on the cycle period. In contrast when the stimulus frequency was set at 150 Hz (B) there was a marked effect.

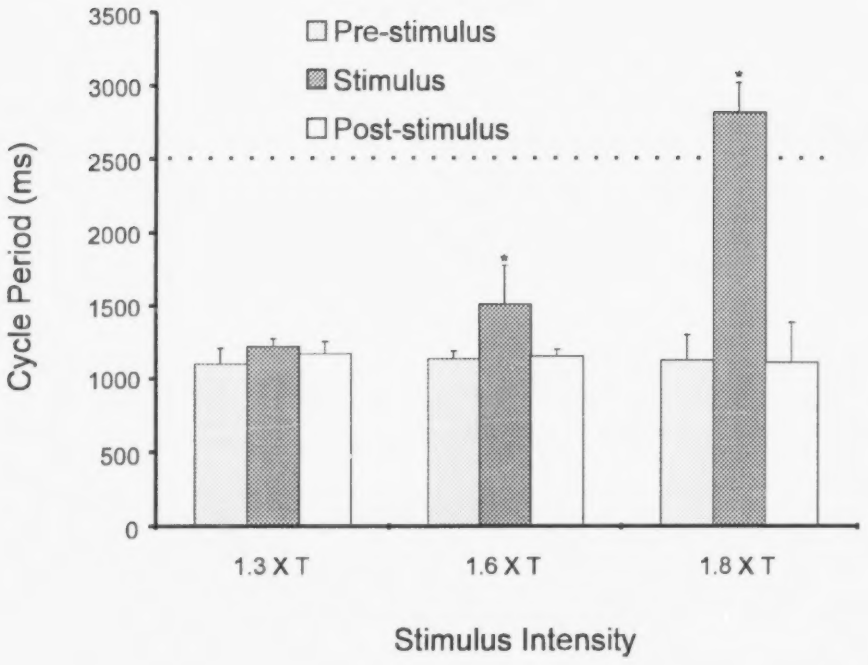


Figure 2-6: Activation of most of the group I afferents was necessary to produce an effect on the locomotor rhythm. The bars indicate average (1.3xT n=4; 1.6xT n=5; 1.8xT n=5). St step cycle durations before (left bar in each series), during (middle bar in each series) and immediately after (right bar in each series) a stimulus train (standard deviation bars indicated for each trial; * = statistically different from control steps ($p<.05$)). The dotted line indicates the average stimulus offset time for the stimulated trials. Stimulus trains were applied to the LGS nerve (trains: 2000ms duration, 150 Hz, 200ms delay). Note that the stimulus had to be increased to 1.8xT before the flexor burst was delayed to the end of the stimulus train.

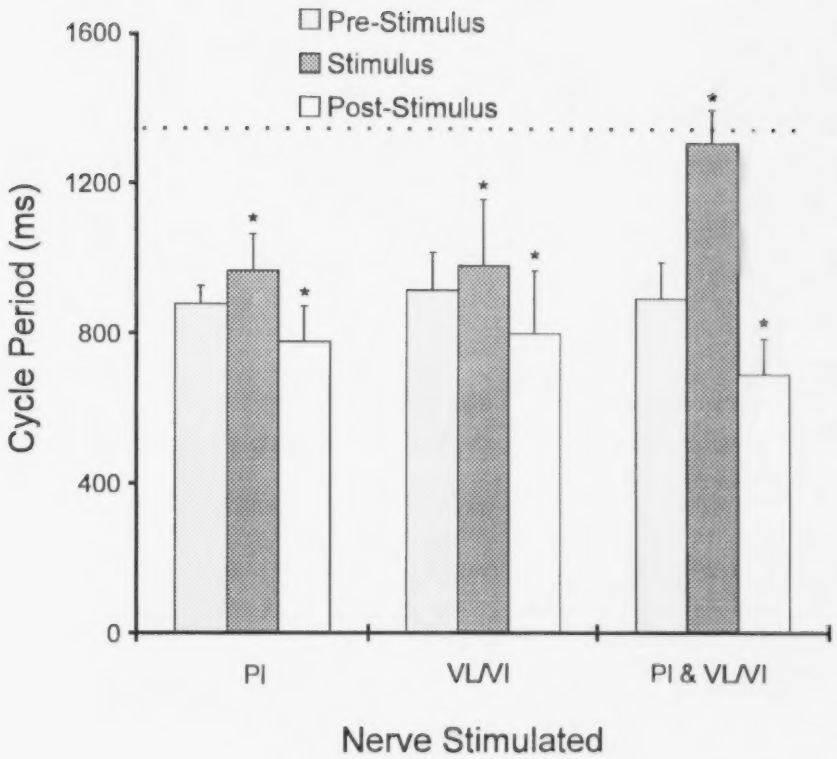


Figure 2-7: Nonlinear summation of effects on locomotor rhythm produced by simultaneous stimulation of the Pl (1.8xT) and VL/Vl (1.8xT) nerves. The bar graph illustrates the average (Pl n=21; VL/Vl n=22; Pl/VL/Vl n=18) St cycle duration immediately before (left bar in each series), during (middle bar in each series), and immediately after (right bar in each series) stimulation (trains: 800ms duration, 150 Hz, 200ms delay). The bar series on the left shows a modest effect on stimulating the Pl nerve alone, similarly the bar series in the middle shows a similar modest effect for VL/Vl. When the two nerves were stimulated simultaneously the cycle period was markedly increased (standard deviation bars are shown for each trial; * = statistically different from control steps ($p<.05$))

a similar latency to LGS stimulation, however, the magnitude of the excitatory response was less (not shown)

When the VL/VI nerve was stimulated an excitation of the VM muscle occurred with a latency of 40-50ms. Although monosynaptic connections occur between VL/VI and VM, the latency suggests that the functional reflex is mediated via polysynaptic pathways. The response onto the triceps surae from VL/VI stimulation was more variable. In 3 animals, stimulation of VL/VI prolonged the MG burst but the excitation only became apparent at the end of the falling phase of the burst. In one animal the MG burst was inhibited during stimulation of VL/VI, the excitation occurred mainly in the knee extensors. In one animal stimulation of VL/VI had no effect on the magnitude of the MG burst, yet delayed the onset of the flexor burst.

Influence of the group I afferents on the flexor phase of the step cycle.

In fictive preparations, it has been shown that group Ib stimulation during flexor activity can cause a disfacilitation of the flexor motoneurones, and cause a concomitant depolarisation of the extensor motoneurones (Conway et al. 1987). Therefore, we were interested in observing whether the group I stimulation would also inhibit flexor burst generation during walking. In 3 cats, the stimulus of the LGS and Pl nerves was triggered off the burst in the flexor muscle Ip at preset delays. Stimuli applied during early flexion had the effect of prematurely terminating the Ip burst and causing a generalised excitation of the ipsilateral extensors (figure 2-9). Ipsilateral flexion was delayed until after the stimulus train ended and the contralateral limb hyperextended to support the animal while the ipsilateral limb flexed. Summation from Pl and LGS was always needed to produce the resetting to extension. If the nerves were stimulated individually, the magnitude of the Ip burst was reduced when compared to normal trials, but the swing phase was

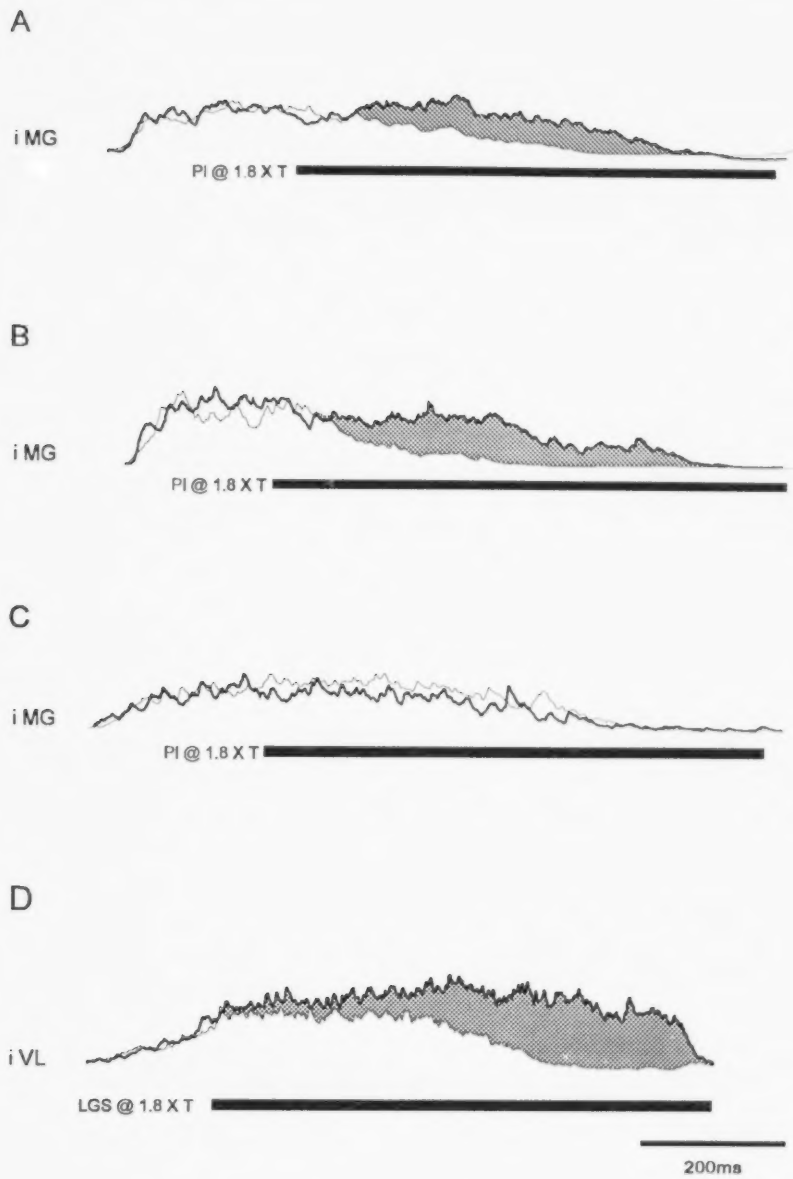


Figure 2-8: A), B), and C). Excitatory actions of PI group I afferents on MG bursts during walking. Rectified and filtered EMG responses from two different animals after stimulation (trains: 800ms, 200 Hz, 200ms duration, 1.8xT). A) and B) show examples of an excitatory effect (shaded region) that occurred in one animal during stimulation (heavy lines = stimulated step cycle, light line = normal step cycle). The excitatory effect was especially evident during the falling phase of the MG burst. C) This example from another animal showed no effect on MG amplitude due to PI nerve stimulation. D) Excitatory actions of LGS group I afferents (trains: 800ms, 200 Hz, 200ms, 1.8xT) on VL bursts during walking. Note that a late excitation occurred after the LGS stimulus onset.

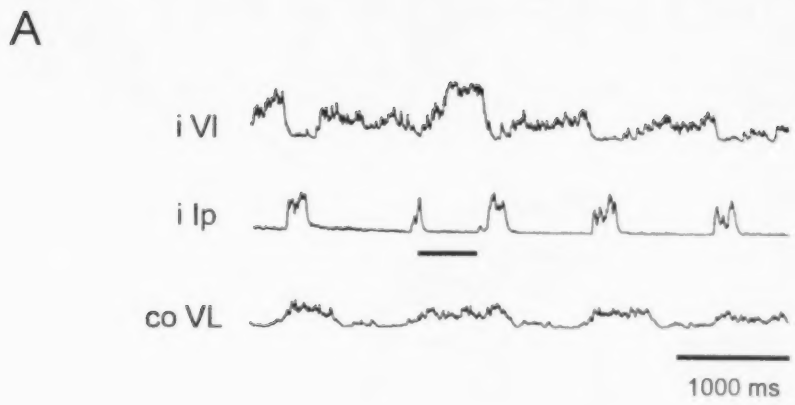
not delayed. Termination of flexion did not occur when the stimulus train was delayed more than 40ms after the onset of Ip burst activity, but the magnitude of the Ip burst was reduced compared to normal bursts.

Discussion

The main result of this study is that electrical stimulation of the group I afferents in nerves to hind leg extensor muscles can prolong the stance phase during walking in decerebrate cats. Although it is difficult to ascribe this effect to either the group Ia or the group Ib afferents by using electrical stimulation, previous studies in spinal preparations (Conway et al. 1987; Gossard et al. 1994; Pearson et al. 1992; Pearson and Collins 1993) suggest that it is largely due to activation of the group Ib afferents.

Our finding of a strong influence of the extensor group I afferents on the locomotor rhythm during stepping is contrary to an earlier conclusion that these afferents have little effect (Duysens & Stein, 1978). We attribute the different results of the two studies to differences in the frequency of the stimuli. When we used the same frequency used by Duysens and Stein (60Hz) we observed no significant effects on the cycle period (figure 2-5A). However, strong effects were observed at frequencies of 150Hz (figure 2-5B). The minimum frequency needed to significantly prolong the cycle period was about 100Hz. The likely reason why 60 Hz stimulation has no effect on the locomotor cycle is that it is well below the GTO firing rate during walking (Ib mean rate 160 Hz; Appenteng and Prochazka, 1984).

Because stimulation of extensor nerves causes a reflex excitation of synergists which act to extend the hindlimb, it is possible that the added extensor thrust onto the moving belt could activate the afferents in the paw responsible for the 'extensor thrust reflex' (described by Sherrington 1947), and this, in turn, could prolong the step cycle. However, this interpretation is



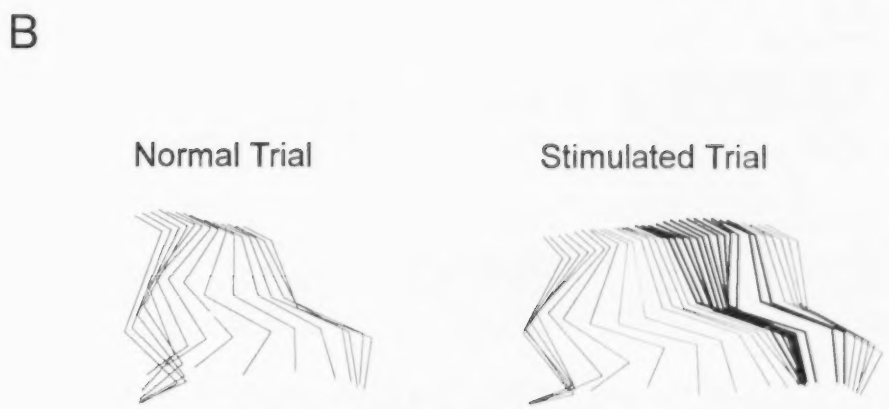


Figure 2-9: Stimulation of extensor nerves during flexion can reset the ipsilateral step cycle to extension. A) Rectified and filtered EMG responses. A) Simultaneous stimulation of the LG-Sol and Pl nerves at 1.8 times threshold. When the stimulus (trains: 600ms duration, 150 Hz, delay 10ms) was applied early during the Ip burst (top traces), it resulted in premature termination of the Ip burst. Note the marked delayed excitation of the ipsilateral VL, and the increase in the duration of the contralateral extensor to support the body during the delayed flexion response. B) Stick figures graphically comparing the normal step cycle (top) with the perturbed step cycle (Bottom). The time of stimulus application is indicated by the black lines. Note the stimulus terminated flexion

unlikely for two reasons. Firstly, qualitatively similar extensor responses were observed in the absence of ground support; in this situation the stimulus train was delivered when the foot entered a hole cut in the treadmill belt (Hiebert et al. 1995). Secondly, Sherrington described the 'extensor thrust reflex' as lasting for a maximum of 200ms and our results show that the prolongation of extension would frequently last for the duration of the stimulus train (up to 2000ms).

An important aspect of our observations was that stimulation of each of the three nerves produced a prolongation of the stance phase and delayed the onset of flexor burst activity (figures 2-1, 2-2). Stimulation of a single nerve typically produced a generalised excitation of the extensor muscles in the ipsilateral hindlimb. Thus, group I afferents in all three nerves must have an excitatory action on the system of interneurons generating the stance-phase extensor activity (we will refer to this system as the "extensor half-centre" [Lundberg 1969,1980]). Interestingly, the effects of stimulating two nerves simultaneously summated, sometimes nonlinearly (figure 2-7). This suggests that at least a portion of the group I afferent input from many extensor muscles converge onto a common interneuronal pool. Our finding of spatial summation differs from results in acute DOPA-treated spinal cats where in most instances there was no spatial summation of excitatory input to extensor motoneurons with simultaneous stimulation of different combinations of ipsilateral extensor nerves (Gossard et al. 1994).

Another characteristic of the excitatory effect of the extensor group I afferents on the cycle period was that the locomotor rhythm was usually not reset, i.e. there was a shortening of the cycle immediately following the prolonged cycle (figures 2-1, 2-2). The contralateral leg showed only a modest increase in the cycle period, and this increase was typically abolished in cases where we could suppress ipsilateral flexion for more than one step cycle. These

observations also differ from findings in acute spinal animals where the locomotor rhythm is consistently reset by stimulation of the extensor group I afferents (Conway et al. 1987; Pearson et al. 1992). The most probable reason for this difference is that during walking in the decerebrate cat there are stepping movements in the other three legs, and the timing of these movements is regulated by the speed of the treadmill (Grillner & Rossignol 1978). If all four rhythm generating networks are fairly tightly coupled (Orsal et al. 1990), then timing of activity in the experimental leg (left hind leg) will be dominated by the interactions with the other three networks and only transiently influenced by afferent feedback. In support of this idea we observed that after perturbation of the ipsilateral leg by extensor nerve stimulation, the ipsilateral rhythm tended to resume with a timing synchronised to the relatively unperturbed contralateral leg.

Previous investigations on spinal animals (L-DOPA and Clonidine treated) have shown that during locomotor activity stimulation of group Ib afferents in the Pl nerve has an excitatory action on MG motoneurons (Pearson and Collins 1993; Gossard et al. 1994). Under resting conditions the effect is inhibitory. In this investigation it was observed that stimulation of the Pl nerve could also produce an additional excitation of MG motoneurons. Consistent with the results from spinal animals, the minimum latency of the excitatory effect was about 50ms. We attribute this long latency to the slow rate of depolarization evoked in MG motoneurons when group Ib afferents are stimulated (Gossard et al. 1994). In our experiments the excitatory action of the Pl group I afferents was most pronounced near the end of the MG bursts when the intensity of activity was falling (figure 2-8A, 2-8B). If during the early part of stance the extensor half-centre is receiving a powerful activating drive then the excitatory actions of any afferent input onto the system would be attenuated. This could explain why the excitatory action is more apparent when the level of MG activity is low near the end of stance, and it is consistent with data from intracellular recordings in L-DOPA treated cats showing only very weak excitatory

actions of Ib afferents on MG motoneurons during the peak of extensor activity (Gossard et al. 1994). If group Ib afferents from all leg extensor muscles have an excitatory action on the extensor half-centre (as proposed in earlier studies [Pearson and Collins 1993; Gossard et al. 1994]) then we would expect that stimulation of the group I afferents in any extensor nerve should produce excitatory actions on the activity of all extensor muscles of the leg. This would add to any excitatory effects produced by monosynaptic homonymous and heteronymous group Ia connections. In accord with this prediction we found that the knee extensor (VM and VL) muscles were excited by stimulation of the LGS and Pl nerves (there are only weak monosynaptic group Ia connections from LGS and Pl to VM or VL) and that stimulation of the LGS nerve generally produced an excitatory effect on VM/Vl (again there are only weak monosynaptic Ia connections from LGS to VM/Vl). In all these cases the minimum latency of the excitatory effect was between 40 and 60ms, with stronger effects occurring later.

In fictive preparations, stimulation of group I extensor nerves during the flexion phase results in a resetting to extension (Conway et al. 1987). Similarly in a walking decerebrate cat, stimulation of the extensor group I afferents results in termination of flexion and a resetting to extension (figure 2-9). In contrast with the fictive preparation, two extensor nerves had to be activated to terminate flexion. If only one extensor nerve was stimulated it caused a reduction in the magnitude of the flexor burst but did not alter the stepping pattern. Another difference was that if the delivery of the stimulus train was delayed more than 30ms into the flexor burst, the magnitude of the flexor burst was reduced, but the stepping pattern was unaffected. One could explain these results by an attenuation of extensor group I afferent transmission caused by increased presynaptic inhibition of the afferent terminals during flexion (Gossard et al. 1994). An alternative explanation for these results relies on the concept of mutually inhibiting half-centres for locomotion (Lundberg 1969, 1980). During the swing phase the flexor half centre is

strongly activated and inhibits the extensor half-centre. Thus, even though the extensor group I afferents are contributing EPSP's to the extensor half-centre network these effects are being cancelled by IPSP's arising from the strongly active flexor half-centre.

What implications do our data have for understanding the function of group I afferents in the regulation of stepping? In answering this question we will assume that the actions we have described in this paper are due primarily to input from the group Ib afferents. We have not excluded the possibility that the group Ia afferents could have similar, but weaker, actions. Indeed, recent evidence shows that short trains of stretches to the triceps surae, which preferentially recruit the group Ia afferents, can enhance extensor activity (D. McCrea, Personal Communication). Previous studies have suggested two functions for the group Ib afferents during walking. The first is to regulate the duration of the stance phase. The proposed mechanism underlying this function is that a decline in the level of activity of the group Ib afferents during latter stance removes inhibition from the flexor burst generating system. The second possible function is that positive feedback from the group Ib afferents during stance reinforces the activity in extensor motoneurons to regulate the level of activity in motoneurons according to the load carried by the leg (Pearson and Collins, 1993). Our data are consistent with both hypotheses: stimulation of the group Ib afferents can delay the onset of flexor burst activity (figures 2-1, 2-2) and can increase the magnitude of burst activity in extensor motoneurons that receive only weak monosynaptic connections from group Ia afferents in the stimulated nerve (figure 2-8). However, the consistency and the size of the effect on extensor burst duration suggest that the primary function of the group Ib afferents is to regulate the duration of the stance phase.

We conclude that stimulation of ankle extensor nerves at group I strengths, contrary to the results of Duysens and Stein (1978), can prolong the extensor burst and delay the onset of

flexion in a walking cat. This observation is consistent with the notion that a necessary condition for the initiation of swing is unloading of extensor muscles as the stance phase progresses. However, it is unlikely that this signal alone is sufficient for initiating the swing phase. Input from the hip afferents have been implicated, and cutaneous signals could be involved since afferents from these receptors have a powerful effect on timing (Duysens 1977; Duysens and Stein 1978). Probably numerous groups of afferents are involved in the stance to swing transition, with the relative importance varying according to the behavioral state of the animal.

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Chapter 3

Stimulation of the group I extensor afferents in the intact walking cat can control the duration of the extensor burst in a task-dependent manner[3]

Introduction

It is generally recognized that the basic locomotor pattern in the cat is produced by neural networks residing wholly within the spinal cord (Grillner, 1981; Rossignol, 1996). However, sensory feedback is an essential component of the locomotor system, as it helps stabilize the locomotor rhythm (Grillner and Zangger, 1984) and also adjusts its output to cope with the various terrains faced by the animal (Grillner , 1985). Chronic spinal cats which have been trained to walk on a treadmill rely totally on afferent feedback to modulate their stepping patterns and can adjust their gait and cadence in sophisticated ways. For example, chronic spinal cats can support their own weight during stepping, adjust their cadence to the speed of the treadmill and even show successful coordination of the two hindlimbs when each leg is driven at a different speed by a split treadmill (Barbeau and Rossignol, 1987). These findings have led to a number of questions regarding the afferent control of locomotion. Firstly, which reflex pathways contribute to the control of locomotion? And secondly, in what way do they contribute during walking in the intact animal?

It has been established recently that afferent signals from sensory receptors located in extensor and flexor muscles can affect the stance to swing transition (Chapter 2 and 3; Hiebert et al.,

[3] This chapter has not been published. We intend to submit this paper for publication in the Fall of 1996. As primary author of this paper, I was heavily involved in the planning, performance, and execution of each experiment and I performed all of the subsequent data analysis. I wrote the initial draft of this document which was edited by Dr. K.G. Pearson for style and content.

1996). During walking electrical or mechanical activation of the flexor muscle spindle afferents (the group Ia and II afferents) can inhibit the extensor bursts and initiate a premature onset of the flexion phase in decerebrate cats (Hiebert et al., 1996). Thus, during walking, signals from the flexor muscle afferents may act to prevent an excessive limb extension during which the limb would not be providing any real support or propulsive force (Grillner, 1985).

In recent years it has been established that activation of group I afferents from muscle spindles and Golgi tendon organs of extensor muscles can also affect the timing of the locomotor rhythm (Conway et al., 1987; Pearson et al., 1992; Pearson and Collins, 1993; Guertin et al., 1995). Furthermore, a recent study has demonstrated that stimulation of extensor group I afferents can increase the duration of the stance phase in the decerebrate cat walking on a motorized treadmill (chapter 2). An important issue arising from these observations is whether feedback from extensor group I afferents also regulates stepping in intact animals. This may not necessarily be the case. Different reflex pathways could be selected by the intact cat to provide timing cues during locomotion or indeed a greater emphasis on descending commands which integrate visual and somatosensory cues could occur (Drew, 1991).

The main question addressed in the current investigation was whether stimulation of extensor group I afferents would prolong the extensor burst and delay the onset on flexor activity in intact walking cats. To test this hypothesis we trained intact cats to walk quadrupedally and bipedally on a motorized treadmill. During mid-stance we stimulated the various extensor nerves using implanted stimulus cuffs at group I strengths and measured the effects on the duration and the amplitude of the extensor bursts. In the course of these studies we had the opportunity to assess whether the effects were dependent on the type of locomotor task performed by the animal. Task-dependency of reflex pathways is well-documented especially in humans (Prochazka, 1989) but

there is little research on the modulation of proprioceptive reflexes in intact cats. Generally, the results supported our hypothesis, however there were some unexpected findings. In the intact animal the magnitude of the effects on the duration and the amplitude of the extensor burst evoked by stimulation of the extensor group I afferents were smaller and more variable than expected.

Methods

All animals used in this study were cared for in accordance with the guidelines published by the American Physiological Society, and the experimental procedures were approved by the University of Alberta animal welfare committee. Experiments were conducted on 12 intact adult cats of both sexes. Of these 12 animals, 3 were rejected due to failure of the stimulating cuff.

Under halothane gas anesthesia, stimulating cuffs were implanted on the lateral gastrocnemius and soleus (LGS), vastus lateralis and vastus intermedius (VL/VI) and plantaris (Pl) nerves in a total of 12 animals (Table 3-0). For convenience, only the successful animals are considered from this point.

Nerve cuffed	LGS only	LGS + Pl	LGS + VL/VI	Pl only	VL/VI only	Rejected Animals	Total Animals
# of cats	5	1	1	1	1	3	12

Table 3-0: Number of intact animals used and type of extensor nerves stimulated

Depending on the animal, the nerves supplying either the LGS, and/or Pl, and/or VL/VI muscles were exposed and transected close to the muscle. A 1 cm length of each nerve was freed and tied into a small bipolar stimulus cuff. The latex stimulus cuffs were approximately 6mm in length

and had an internal diameter of about 3 mm. The bared ends of two Teflon-coated stainless steel wires (AS631, Cooner wire company) were looped within the cuffs and acted as the stimulus electrodes. To record the afferent volley from the LGS or Pl nerve, a recording cuff was placed around the sciatic nerve just distal to the hamstrings nerve junction. In the 2 animals with VL/VI stimulating cuffs we monitored the volley by recording a cord dorsum potential. A laminectomy was performed at the level of L4-L5 and the lamina between the two spinous processes were removed. Recording wires (AS632, Cooner Wire Company) were placed on the dorsal surface of the spinal cord just underneath the laminae. The wires were held in place by inserting two small pins into each of the L4 and L5 spinous processes and fixing the wires in place by applying a small amount of dental acrylic to each spinous process. The threshold of the electrical stimulus to the extensor nerve was taken as the minimum voltage necessary to produce a just detectable potential from either the sciatic nerve or the cord dorsum. The strength of the electrical stimulus to the extensor nerve was expressed in multiples of this threshold level. Bipolar stainless steel electrodes were sewn into the various combination of the following muscles of the experimental leg to record the electromyographic (EMG) activity during walking: medial gastrocnemius (MG), vastus lateralis (VL), vastus medialis; semi-tendinosis (St), tibialis anterior (TA), lateral gastrocnemius (LG). In addition, EMG recording electrodes were implanted in combination of the following contralateral muscles: St, MG, TA, or VL. The wires from both the recording and stimulating electrodes were led subcutaneously and connected to a socket that was imbedded into a headpiece manufactured from dental acrylic. After this procedure, the cats were administered a dose of an antibiotic (Amoxicillin, .50 mg) and an analgesic (Buprenorphine, 0.005-0.01 mg/kg) and were allowed to recover for 1-2 days. In one animal the implantation of the EMG electrodes, sciatic recording cuff, and the headpiece was completed one week prior to the actual implantation of the LGS stimulating cuff. During the second surgical procedure, the cuff was implanted onto

the LGS nerve as described earlier. This allowed recordings to be made as soon as 8 hours after the implantation of the cuff.

Recording of data in intact animals

All animals were trained to walk quadrupedally (9 out of 9 cats) and in some cases bipedally (6 out of 9 cats) on a motorized treadmill before the operation took place. The animals were induced to walk by a mixture of food rewards and affection. During the recording of experimental data, kinematic markers were placed on the hip, knee and ankle and walking sequences were recorded using a video camera. During periods of reliable quadrupedal or bipedal walking, a train of stimuli was applied to the LGS nerve during mid-stance (trains 1000 ms duration, 1.8 x T, 200 Hz, 200 ms delay from the onset of the MG EMG). These stimulus parameters were similar to those we had used previously in walking decerebrate cats (chapter 2). Trains (trains 300 ms duration, 1.8 x T, 200 Hz, 0-30 ms delay from the onset of the TA or St EMG) were also delivered during the flexion phase of the step cycle during bipedal (1 animal) or quadrupedal walking (1 animal).

Decerebrate Preparation

To compare the effects of stimulation of the LGS nerve on prolonging the step cycle in intact and decerebrate animals, we decerebrated four of the intact cats in which we had previously recorded data. A minimum amount of dissection was performed and the chronically implanted cuff was used to stimulate the LGS nerve. The decerebration was performed by transecting the brainstem just rostral to the superior colliculi and continuing the cut just rostral to the mammillary bodies, thus producing a premammillary cat (Shik et al., 1966). Approximately 1 hour after decerebration, walking commenced in 3 of the 4 animals. This walking lasted for 1-3 hours (depending on the animal) and occurred in bouts lasting for approximately 5-10 minutes each. The animals walked at a speed that ranged between .25-.35 m/s. During walking, a stimulus train

was applied to the LGS nerve using stimulus parameters that matched those used earlier that day when the animal was intact. In 2 out of the 3 decerebrate cats we implanted an additional stimulus cuff onto the LGS nerve of the contralateral leg. During bouts of walking we compared the effects of stimulating the ipsilateral LGS nerve with those produced by equivalent stimulation of the contralateral LGS nerve to test whether the effects were reduced.

Acute decerebrate cats

These animals (n=4) were used in a separate series of experiments (chapter 5). A precollicular premammillary decerebration was performed on these animals. In these animals the MG and LGS nerves were exposed, transected and fitted with latex stimulating cuffs. In all 4 animals MLR stimulation was used to evoke locomotion (Shik et al. 1966). The stimulating electrode was guided stereotaxically around coordinates P2: L4: H6 until an area was found in which the stimulus trains elicited locomotion with a low stimulus intensity. During walking, the frequency or the voltage of the MLR stimulus train was varied within a set range that produced a walking pattern. The LGS or MG nerve was then stimulated during mid-stance (trains: 1000 ms duration, 200 Hz, 1.8-2 x T) at different intensities of MLR stimulation.

Data analysis

All data were recorded using a Vetter 4000A PCM recorder. Later, sequences were selected by matching the video footage to the EMG traces to determine if the walking pattern was normal. These selected sequences were stored on computer disc using the Axotape (Axon Instruments) data acquisition system installed on a Microexpress 486 computer. Data analyses were carried out using custom programs that could retrieve data from the axotape files. The cycle periods before, during, and after the stimulus were calculated only during regular sequences of walking. Each cycle period was calculated as the time between the occurrence of successive St or Ip bursts. A spreadsheet program (Microsoft Excel 5.0) was used to calculate the mean and standard

deviation for these cycle periods and Student's t-tests were administered to detect significant differences between the conditions. The data were normalised according to the equation below to allow for comparisons between cats.

Equation 1:

Percentage increase in extensor duration = [(b-a)/a] x 100

where *b* indicates the stimulated cycle period and *a* the control cycle period.

Kinematic data, including stick figures, were obtained by analysing selected video sequences using Video Blaster software (Creative Labs) and custom designed software. To analyse significant changes in the amplitude of the extensor EMGs following stimulation of the group I extensor afferents, we employed the following method: the extensor EMG from 5-20 stimulus trials were averaged using custom software and the data was transferred to Microsoft Excel and further analysis. For each trial the prestimulus extensor EMG activity was averaged for 10 ms prior to the onset of the stimulus train.

Results

We previously reported that stimulation of the extensor group I afferents had a strong influence on the duration of the extensor burst in decerebrate cats walking on a treadmill (chapter 2). One of the main questions of the present study was whether similar stimulation of extensor group I afferents could regulate the duration of the stance phase in the intact cat. We addressed this question by stimulating the extensor group I afferents in 9 intact cats walking on a treadmill using similar parameters of stimulation as we used in our previous study.

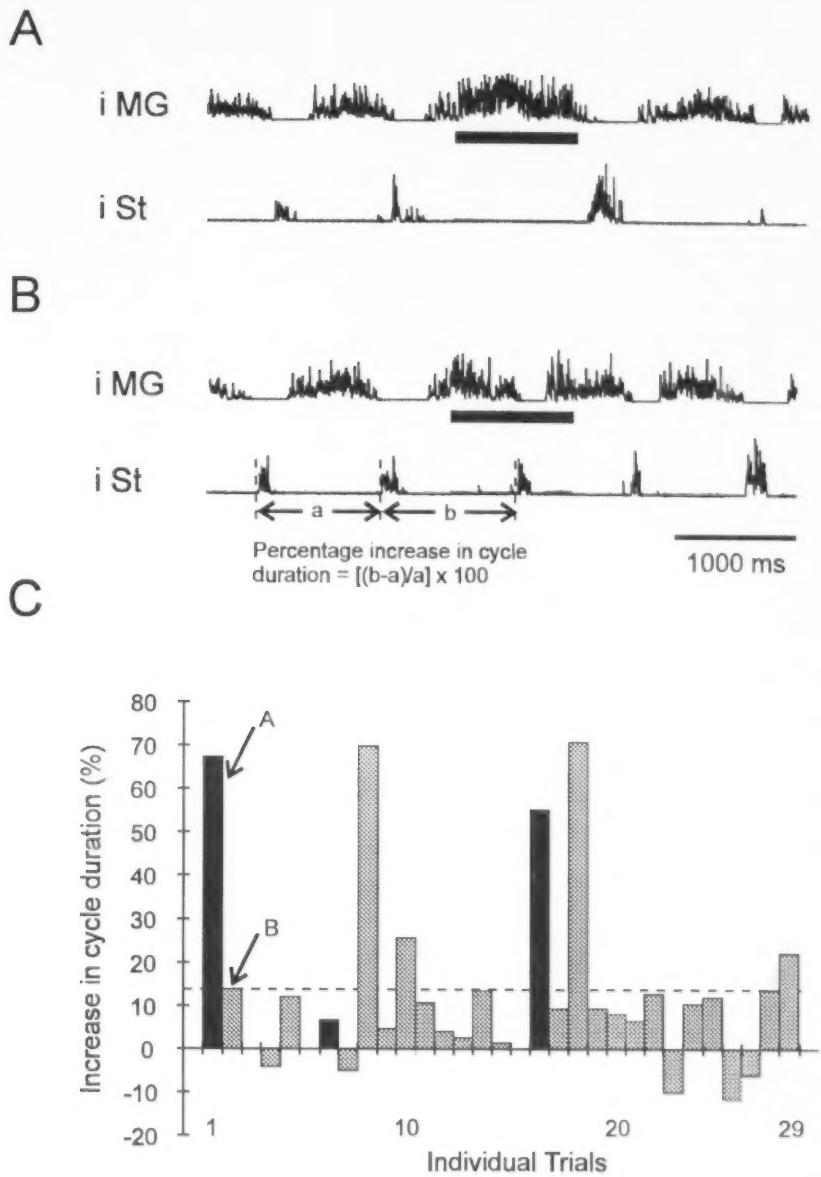

Figure 3-0: Stimulation of the extensor group I afferents can prolong the extensor burst and delay the onset of the flexor burst in the intact quadrupedally walking cat. A,B. Rectified and filtered EMG traces. A. Example of one trial in which stimulation of the LGS nerve (trains: 1000 ms duration; 1.8 x T, 200 Hz) prolonged the MG EMG burst and delayed the onset of the St burst for the duration of the stimulus train. B. The very next stimulus trial produced only a modest effect on the duration of stance. C. Bar graph illustrating the percentage increase in duration of the step cycle following stimulation of the LGS nerve for all the quadrupedal walking trials in a single cat. Each bar represents an individual trial and all data presented were recorded over a period of 4-5 hours. The black bar indicates the start of a continuous stepping sequence. The letters A and B indicate the trials shown in A and B. The dotted line represents the mean increase in cycle duration of all the individual trials shown *(i MG medial gastrocnemius; i St semitendinosis).*

Influence of extensor group I afferents on the duration of extensor muscle activity during intact walking

Initially, in four intact animals we implanted stimulus cuffs onto various extensor nerves (Pl, and/or VL/VI and/or LGS), however we later chose in 5 animals to only implant a stimulus cuff onto the LGS nerve (see Table 3-0). There were two reasons for this decision, firstly we had previously found that stimulation of the LGS nerve had a more powerful effect on the duration of stance in the decerebrate animal than stimulation of the Pl or VL/VI nerves (chapter 2), and secondly we wished to simplify the surgical procedure to allow for a more rapid recovery of the intact animals. Stimulation of either the Pl (one of two animals) or the VL/VI nerves (one animal) at group I strength significantly increased the duration of the extensor burst and delayed the onset of flexion (data not shown; $p<.05$). Since the effects on duration from stimulating the PL and VL/VI nerves were qualitatively similar to LGS stimulation, this section concentrates on describing the results obtained during stimulation of the LGS nerve.

Generally, stimulation of the group I afferents in the LGS nerve (trains: 1000 ms duration, 200 ms delay triggered off the MG burst, 200 Hz, 2 x T) modestly increased the duration of the extensor burst, and delayed the onset of the St burst (figure 3-0B), thus affecting the timing of the stance to swing transition. This occurred in 5 out of 5 bipedally walking and 5 out of 6 quadrupedally walking animals. In one additional animal we stimulated the group I afferents in the LGS and the VL/VI nerve simultaneously during quadrupedal or bipedal walking and again the increase in stance duration was significant ($p<.05$) for both walking conditions.

The effectiveness of extensor group I stimulation in prolonging stance was quite variable from one trial to the next (figure 3-0). Figure 3-0A shows that occasionally (4 stimulus trials out of 29 in this animal), LGS stimulus trains prolonged the extensor burst for the duration of the stimulus train during quadrupedal walking. However, the next stimulus trial following the one shown in

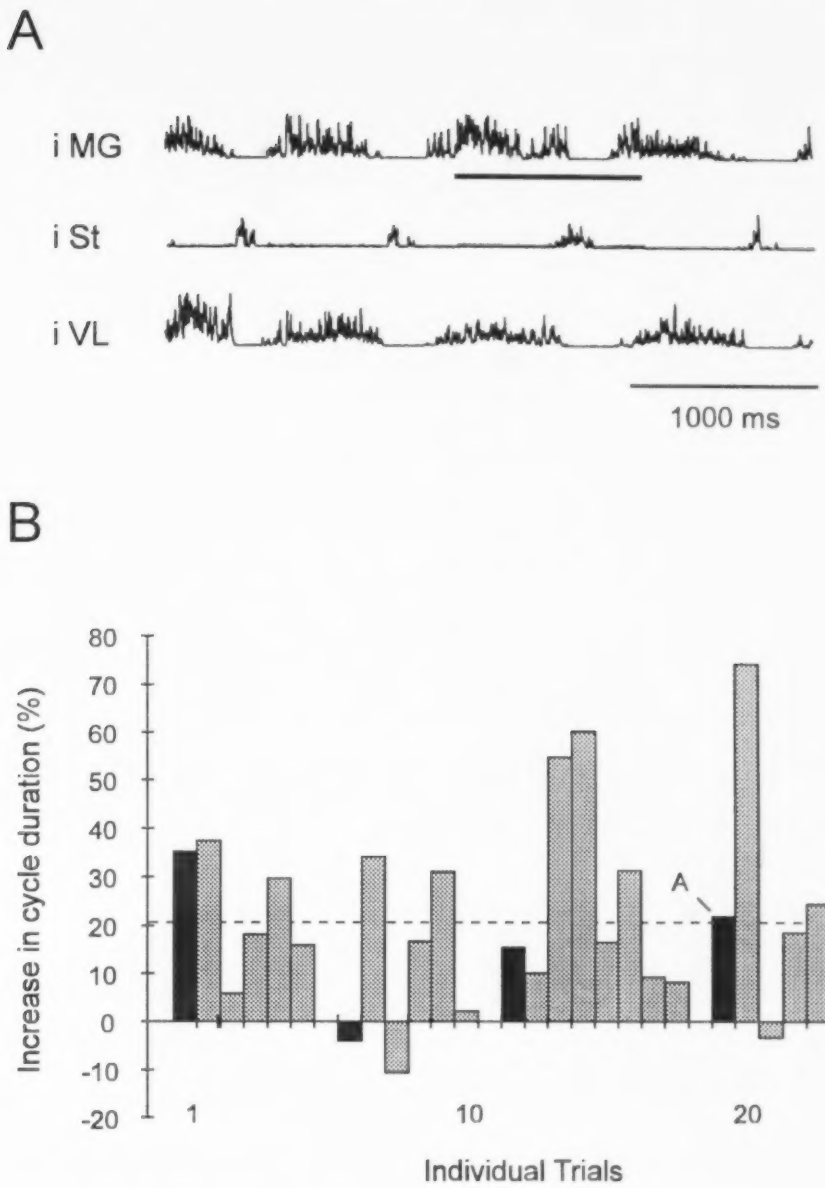

Figure 3-1: Stimulation of the extensor group I afferents prolonged the extensor burst for a greater period of time when the animal was walking bipedally (same animal as in figure 3-0). A. Rectified and filtered EMG traces showing the effects of stimulation of the LGS group I afferents when a cat was walking bipedally. B. Bar graph illustrating the percentage increase in duration of the step cycle following stimulation of the LGS group I afferents for all the bipedal walking trials obtained from a single cat. Each bar represents an individual trial and all data presented were recorded over a period of 4-5 hours. The black bar indicates the start of a continuous stepping sequence. The capital letter (A) indicates the trials illustrated in A. The dotted line represents the mean increase in cycle duration of all the individual trials shown.

figure 3-0A (figure 3-0B) only modestly increased the duration of the extensor burst illustrating how variable the effects could be. When the individual trials from this animal were examined, two features of the data were apparent (figure 3-0C). Firstly, most stimulus trials increased the step cycle duration by less than 14%, and, secondly there was a trend for stimulus trials at the start of each recording session to have a greater effect than trials at the end (in figure 3-0C the beginning of each recording session is marked by a black bar - all data shown in this figure were collected over a period of 4-5 hours). A reduction of the effects of stimulating the extensor group I afferents was observed in three additional animals following a number of stimulus trains. In the remaining animals, although the effects of stimulating the LGS nerve had variable effects, there was no tendency for a decrease in the effectiveness to occur over time.

Generally, stimulation of the extensor group I afferents affected both the ipsilateral and the contralateral step cycle. When stimulation of group I extensor afferents delayed the onset of the ipsilateral swing phase, the contralateral stance phase was always increased to support the animal's weight. The pattern of stepping would usually return to normal one to two step cycles following the perturbed step (data not shown). In decerebrate walking animals we reported that the contralateral hindlimb would normally continue to step at the same rate during stimulation of the group I afferents in the LGS nerve, while the ipsilateral extensor burst was prolonged for the duration of the stimulus (chapter 2). It was possible for the contralateral hindlimb to continue to step in the decerebrate cat since the weight of the animal was partly supported by a harness. This type of adaptation presumably did not occur in intact quadrupedally walking animals because it would lead to instability. However, in one animal walking bipedally, the LGS stimulus occasionally held off stance for the duration of the stimulus train. This animal compensated for the increase in ipsilateral stance duration by making a quick double step of the contralateral leg (contralateral movement was monitored using video in this animal).This type of compensation

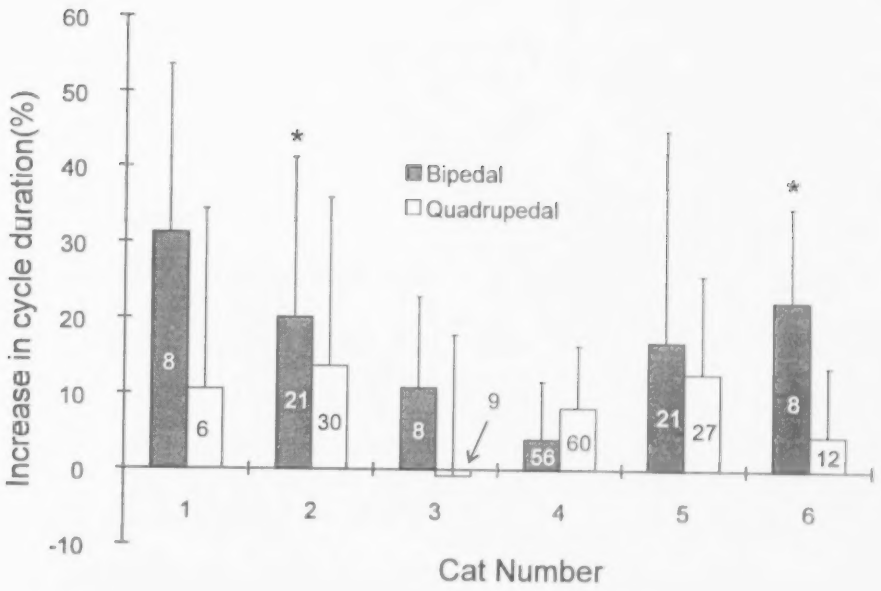

Figure 3-2: Stimulation of the LGS nerve at group I strengths during bipedal walking prolonged stance to a greater degree than when cats were quadrupedally walking. The bar chart shows the mean increase in the duration of the cycle period following stimulation of the LGS nerve during bipedal (gray bars) and quadrupedal walking (white bars) for each animal tested. The error bars show the standard deviation, while the numbers inside the bars indicate the number of individual trials used to calculate the mean. An asterisk above each bar indicates that the difference was statistically significant ($p < .05$).

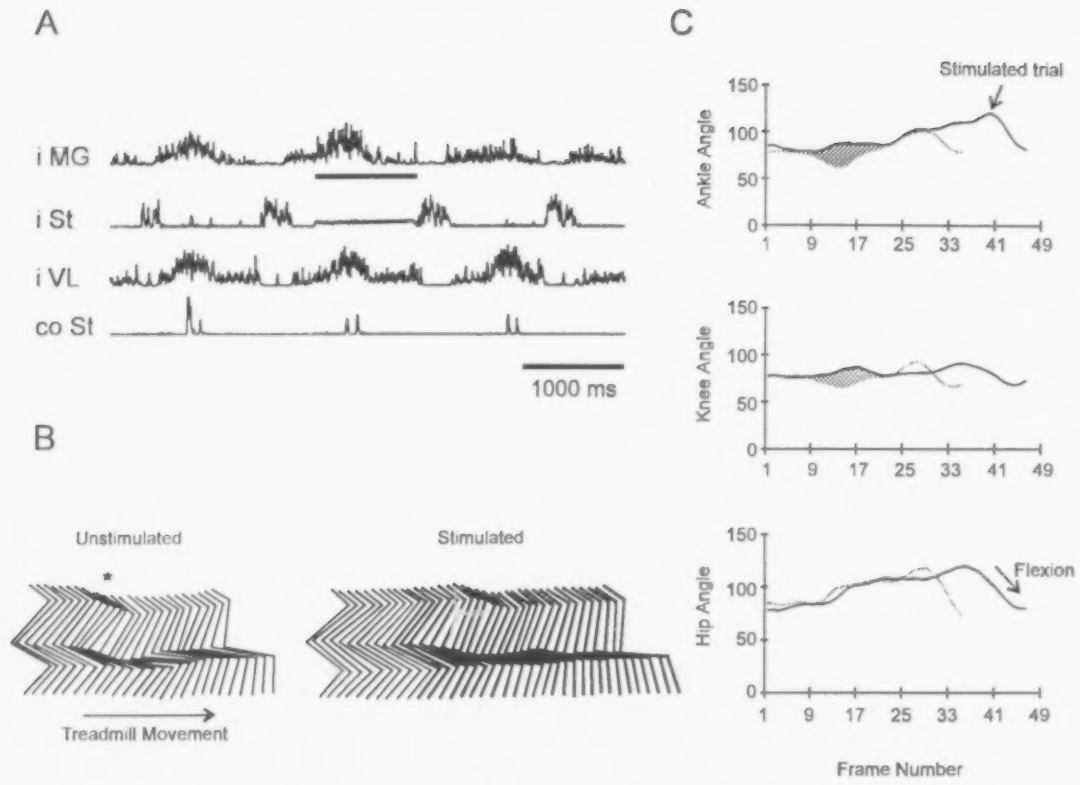

Figure 3-3: Stimulation of the LGS group I afferents increases the MG EMG and abolishes the yield. A: Rectified and filtered EMG responses from muscles of the hindlimb during walking. Stimulus trains were delivered to the LGS nerve and triggered off the MG burst (train: 1000 ms duration, 200 Hz, 200 ms delay following the start of the MG burst). Note the increase in the MG amplitude and that the iSt burst was delayed for the duration of the stimulus train. B. Stick figure of the same sequence shown in A. The heavy lines (in the stim stick figure) indicate when the stimulus train was on. Note that in the unstimulated trial there was an pronounced yield of the knee and ankle (marked with the asterisk) during mid stance due to axotomy of the LGS nerve 1 day prior to the recording session. This yield of the ankle and the knee were eliminated during LGS stimulation. C. Plots of the ankle, knee and hip angles taken from the stick figure shown in B, clearly illustrating the yield in the ankle and the knee during unstimulated trials and the elimination of this yield during LGS stimulation (gray shaded regions). Note that the ankle tended to hyperextend at the end of the stimulus train, while the hip and knee angles were relatively unchanged. The frame number on the abscissa relate to the individual video frames used to generate the individual stick figures. *(i MG ipsilateral medial gastrocnemius, i St ipsilateral semitendinosis, i VL ipsilateral vastus lateralis, co St contralateral semitendinosis).*

was not observed during quadrupedal walking. Nevertheless, on some occasions stimulation of the LGS afferents did prolong stance for long periods of time. In these situations the animal's cadence was slowed as a result of an increase in the ipsilateral and contralateral stance phases. Another strategy employed by intact animals (both quadrupedal and bipedal) upon receiving a stimulus that prolonged stance for a long period of time, was to simply stop walking. Presumably, the animals stopped walking so that they could maintain their balance.

We noticed that the ability of extensor group I stimulation to prolong stance varied considerably when the animal was altering its behavior, for example when it was approaching its food dish, looking around, or when it was stopping. These effects were impossible to quantify since we had no control over these behaviors, but they did suggest that the effectiveness of the stimulus was related to the state of the animal. To examine this idea of state dependent changes we made a detailed comparison of stimulating the LGS nerve in the same animal (n=6) during bipedal and quadrupedal walking in the same recording session.

During periods of bipedal walking we noticed that the effects of stimulating the LGS nerve produced more consistent effects on the duration of the step cycle (figure 3-1). Moreover, these effects were often larger than those observed when the animal was walking quadrupedally. Figure 3-2B shows mean data from 6 animals comparing the effects of LGS stimulus trains [trains: 1000ms duration, 1.8 - 2xT] in increasing the duration of the step cycle during bipedal and quadrupedal stepping (comparisons between quadrupedal and bipedal walking, were made only for stimulus trains delivered when the animals were walking at similar cycle periods ($p>.1$)). There was a trend (figure 3-2) for the LGS stimulus trains to cause a greater increase in the average duration of the step cycle during bipedal compared to quadrupedal walking. However, this increase in the duration of step cycle was only significantly different for 2 out of the 6

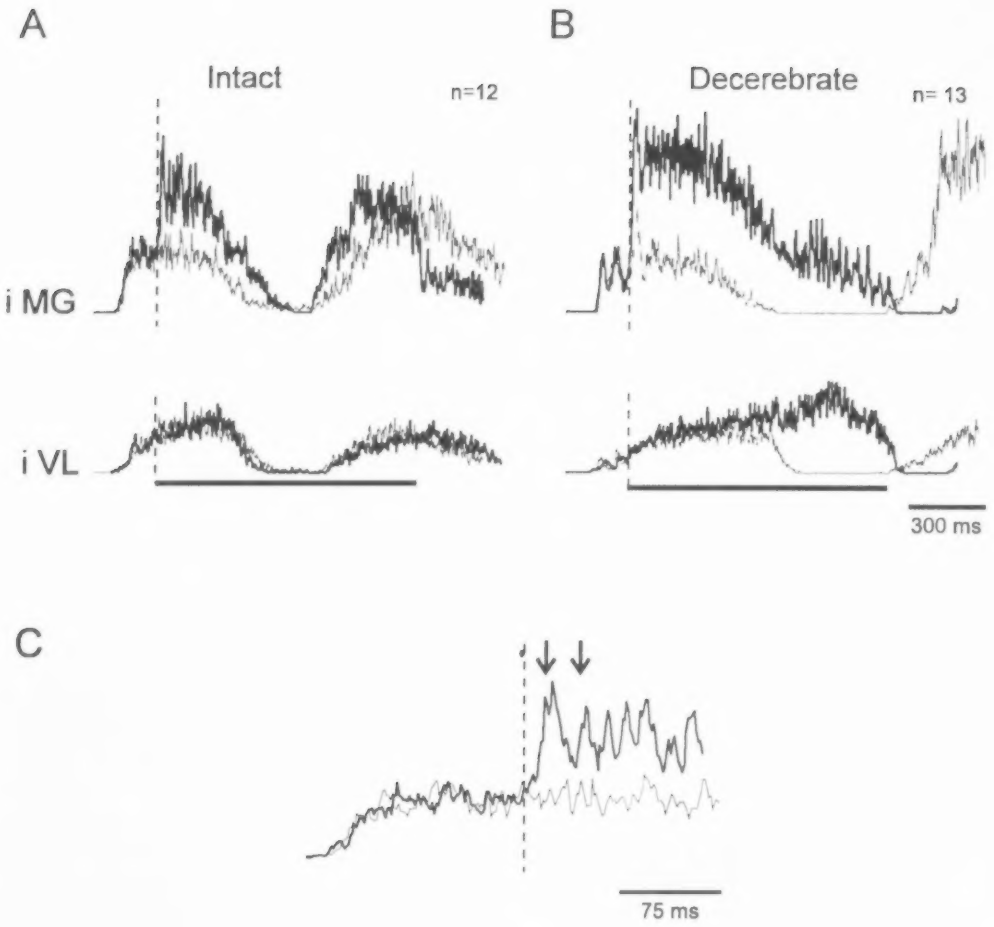

Figure 3-4: Excitatory effects of LGS group I stimulation on the amplitude of the ipsilateral MG and VL in the intact and decerebrate walking state. A,B. Rectified and filtered mean EMG traces. A. LGS stimulation (trains: 1000 ms duration, 1.8 x T, 200 Hz) during intact quadrupedal walking. Note the increase of the MG EMG burst (dark line) compared to the unstimulated burst (light line). B. Similar stimulation 4 hours later when the animal was spontaneously walking on a treadmill after being decerebrated. Note that stimulation of the LGS group I afferents caused a large increase in the amplitude and the duration of the MG EMG burst (dark line). The VL burst was prolonged but the amplitude was not increased until late in the extensor burst. C. Expanded view of the MG burst (from A) showing the presence of two excitatory components produced by LGS stimulation. Stimulus onset is indicated by the dotted line. The amplitude of the MG EMG increased 5-7 ms (first arrow in figure) following the onset of the stimulus train. A later component occurred at a latency of 40-50 ms (second arrow in figure).

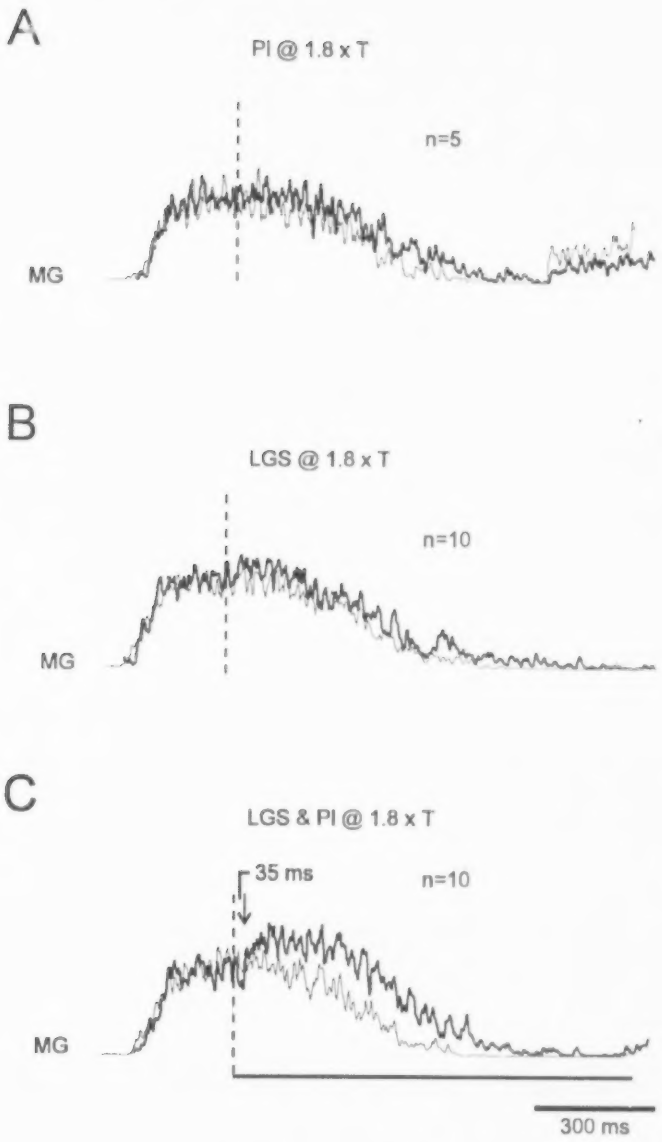

Figure 3-5: Stimulation of two extensor nerves simultaneously could increase the amplitude of the extensor burst in the intact cat. A,B,C Rectified and filtered mean EMG traces from the MG muscle. The dotted line indicates the onset of the stimulus train (trains 1000 ms duration; 1.8 x T; 200 Hz). A. Stimulation of the plantaris nerve did not increase the amplitude of the extensor burst. B. Stimulation of the LGS nerve only modestly increased the amplitude of the MG extensor burst. C. When both the LGS and Pl nerves were stimulated simultaneously a marked increase in the amplitude and duration of the MG extensor burst occurred at a latency of 35 ms following the onset of the stimulus train. The dark line indicates when the stimulus train was on.

animals. When the average data for each animal was pooled, a significant difference was found to exist between the bipedal and the quadrupedal stimulus trials (bipedal walking trials - mean increase 18% ± 10 SD; quadrupedal walking trials 9% ± 6 SD; $p < .05$)

Effects on the amplitude of the extensor burst during stimulation of group I extensor afferents.

During bipedal and quadrupedal walking, LGS stimulus trains increased the amplitude of the extensor burst (figure 3-0A & 3-3A). These increases in the amplitude of the extensor EMG were reflected in the kinematics of the hindlimb (figure 3-3B, 3-3C; data from a bipedally walking animal). Stimulation of the group I afferents in the LGS nerve could eliminate the yield of the ankle and knee that occurred during mid-stance when the contralateral hindlimb entered its swing phase (figure 3-3C). This yield was greater than in normal cats since the LGS nerve was cut, and thus caused a marked drop of the hindquarters during the swing phase of the opposite leg (figure 3-3B). It is likely that activation of the MG muscle contributed to the reduction in the yield during periods when the LGS nerve was stimulated, since the amplitude of the MG EMG was increased (figure 3-3A). The kinematics showed that the yield of the knee was also reduced by LGS stimulation. We were unable to observe any change in the VL EMG that could explain this reduction in the yield of the knee (figure 3-3A and 3-4A).

The changes in the amplitude of the extensor EMGs were most frequently observed in the MG muscle. Figure 3-4A illustrates that the amplitude of the EMG from the MG muscle sharply increased during stimulation of the LGS group I afferents. The increase in the amplitude of the MG burst during LGS stimulus trains had two components (figure 3-4C). The first component occurred at a latency of 7-10 ms consistent with activation of the monosynaptic heteronymous group Ia pathway from LGS or the disynaptic group I pathway (McCrea et al., 1995a). The second component occurred at a longer latency of 35-50 ms and was likely due to activation of

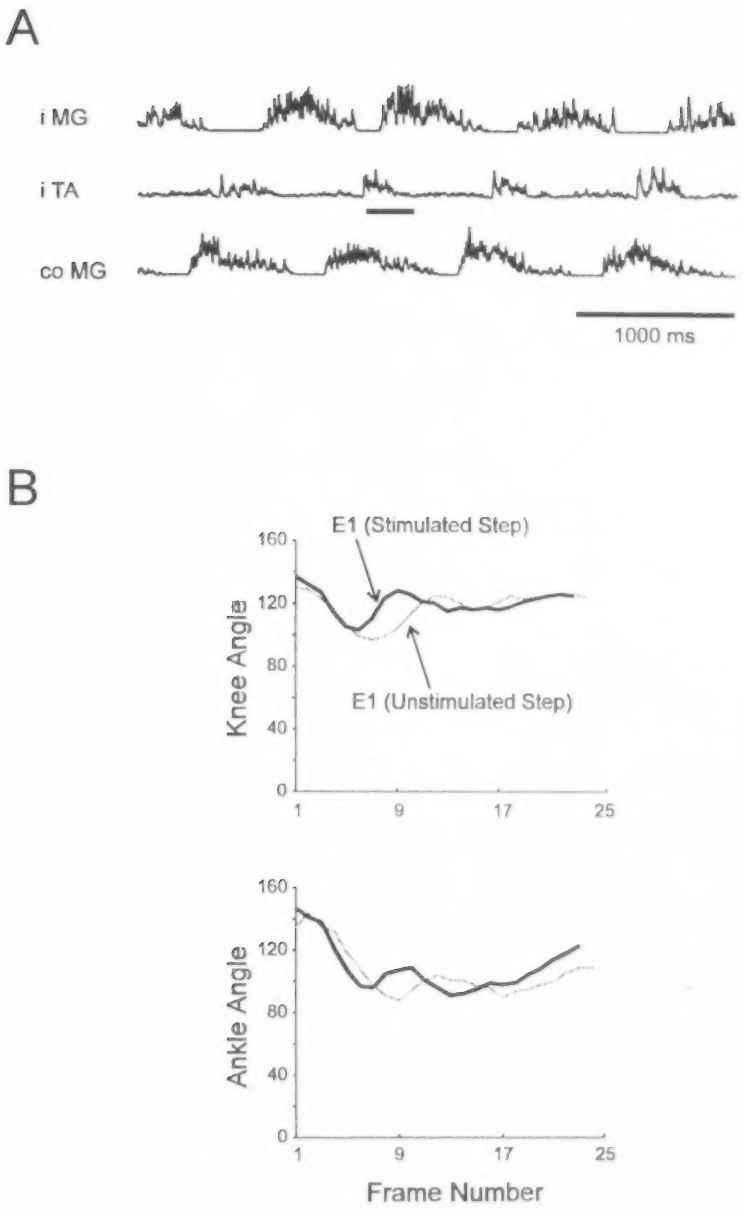

Figure 3-6: Stimulation of the LGS nerve during flexion (trains: 300 ms duration, 200 Hz, 2 x T, 20 ms delay off the TA burst) causes a premature onset of the ipsilateral extensor burst. A Rectified and filtered EMG traces in an intact cat walking bipedally on a treadmill. Note that stimulation of the LGS nerve (black bar) did not affect the TA burst but caused the onset of the extensor burst to occur prematurely. B. Kinematic data (of trial in A) showing changes in the angles around the knee and ankle joint during a single step when the LGS nerve was stimulated at the onset of flexion (dark line) compared to a unstimulated step (light line). Note the earlier onset of the E_1 phase *(i MG ipsilateral medial gastrocnemius, i TA ipsilateral tibialis anterior, co MG contralateral medial gastrocnemius).*

the group I pathways (Pearson and Collins, 1993). In the remaining 3 animals a non-significant increase in the amplitude of the EMG from the MG muscle occurred at either the short (7-10 ms) or long (35-50ms) latencies. Although we recorded the EMG from the quadriceps muscle group in all 6 animals, we never observed any increase in the amplitude of the VL or VM EMG burst.

In 1 intact cat we were able to study the combined effects of stimulating the plantaris and LGS group I afferents on the amplitude of the extensor burst. In this animal neither stimulation of the Pl (figure 3-5A) nor LGS nerves (figure 3-5B) noticeably increased the amplitude of the extensor burst. However, if both nerves were stimulated together a marked increase in amplitude occurred at a latency of 35 ms (Figure 3-5C). In another cat in which only the Pl nerve was stimulated, there was no noticeable increase in the amplitude of the MG EMG during the first 60 ms following the onset of the stimulus. The effects on the amplitude of the MG burst were only apparent during the falling edge of the MG EMG burst.

Although extensor group I stimulation generally increased the amplitude of extensor EMG bursts, stimulus trains occasionally produced inhibitory responses. This inhibition was observed in 2 animals. In the first animal, stimulation of the VL/VI nerve at group I strengths produced an inhibition of the LG EMG accompanied by a non-significant increase in the magnitude of the VM burst (data not shown). In the second animal, stimulation of both the LGS and Pl nerves at group I strengths produced a short latency inhibition of the VM EMG which occurred at a latency of 10 ms.

Effects of extensor group I stimulation applied during flexion

In 2 intact cats, stimulation of extensor group I afferents during early flexion (LGS and Pl nerves in 1 cat; LGS nerve in the other animal; trains: 300 ms duration; 200 Hz, 1.8 x T, triggered 0-50 ms from the beginning of either the TA or St bursts) produced an earlier onset of the next

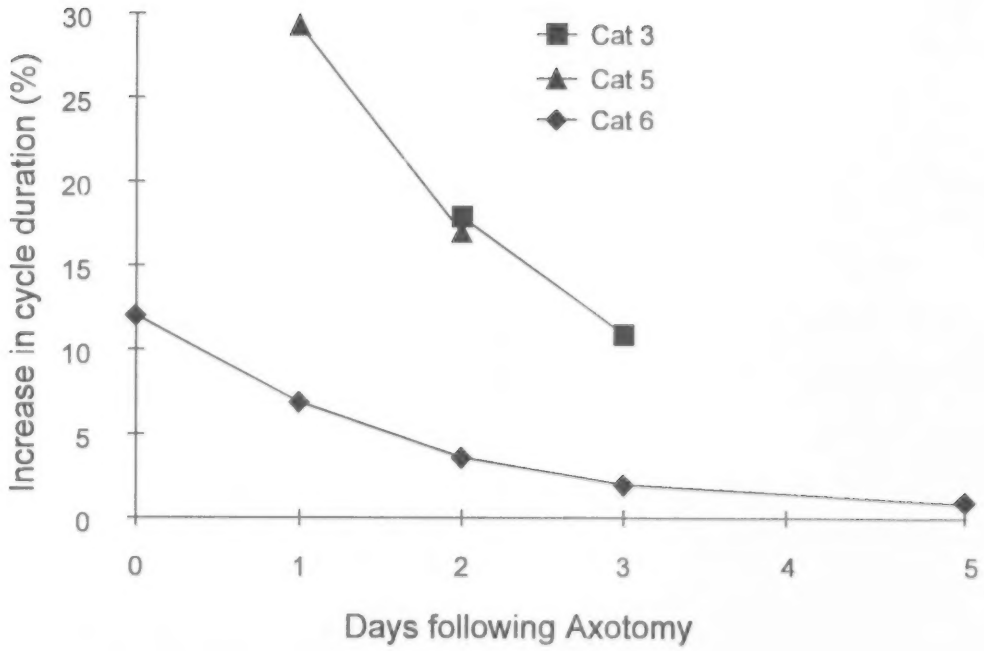

Figure 3-7: The mean effectiveness of LGS group I stimulation in intact animals declined over time. The figure represents the average effects of stimulating the LGS nerve (trains 1000 ms; 1.8 x T, 200 Hz) for 3 animals that were tested for more than one day.

extensor burst (figure 3-6A). This was reflected in the kinematics which showed that the E_1 phase was phase-advanced (figure 3-6C). Overall, there was a mean reduction in the duration of the ipsilateral step cycle as a result of extensor group I stimulation during flexion [n=2: (1) 9% ± 10 SD, (2) 8% ± 10 SD; $p<.05$]. In both animals the amplitude of the flexor burst during extensor nerve stimulation was not noticeably decreased when the stimulus was presented at the onset of the flexor burst.

A decline in LGS effectiveness occurred over time

The effects of stimulating the group I afferents in the LGS nerve progressively decreased from one day to the next (figure 3-7). In 1 animal we were able to observe that the influence of LGS stimulation on prolonging stance gradually decreased over a period of 5 days, with the initial recording being made 8 hours after the implantation of the nerve cuff (figure 3-7, cat 6). One possibility that could explain the reduction in the effects of stimulating the LGS nerve was that the implanted stimulus cuff was damaging the group I afferents. Cuffs are known to damage afferents in nerves by compressing them or by causing a torsion of the nerve as the animal moves (Strain and Olsen, 1975). Large diameter afferents, such as the group I afferents, are more susceptible to injury in both cases (Strain and Olsen, 1975). One observation that suggests that the stimulus cuff did not damage the group I afferents was our ability to record heteronymous H-reflexes from the MG muscle in all 3 of the animals tested over a period of days [stimulus parameters: 1 ms pulse duration, 2 Hz]. If group I afferents were selectively damaged, we would have expected the threshold for eliciting an H-reflex to rise. This did not occur (data not shown).

Decerebration increases influence of LGS group I afferents

Since the influence of LGS group I stimulation declined over time in the intact cat, we were concerned that this could account for the differences in the magnitude of the effects between the intact and decerebrate animal. To test for this possibility we made a direct comparison between

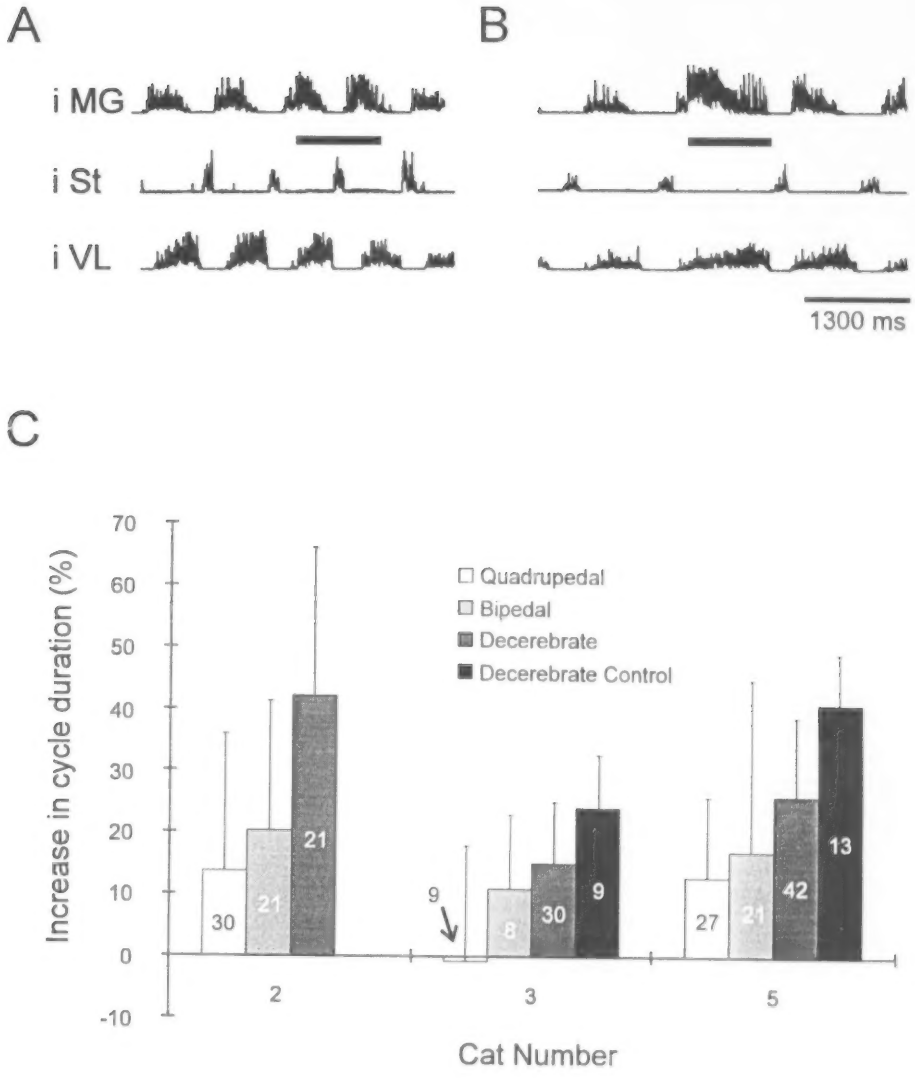

Figure 3-8: LGS stimulation in intact walking animals was less effective in prolonging stance than in the same animals when walking following decerebration. A,B Rectified and filtered EMG traces from a single animal during stimulation of the LGS nerve (trains: 1000 ms duration, 1.8 x T, 200 Hz, train delayed 200 ms from the beginning of the MG burst). When the animal was intact and walking quadrupedally (A) and when the same animal was walking following decerebration (B). C. Bar graph comparing the effects of LGS stimulation during quadrupedal, bipedal and decerebrate walking. In 2 of these animals the effects of stimulation of the LGS nerve in the control leg after decerebration were also recorded (black bars). Note that in all 3 animals stimulation of the LGS nerve during decerebrate walking significantly ($p<.05$) prolonged the step cycle for a greater period of time than similar stimulation during intact walking. Note also that stimulation of the LGS nerve in the control leg (black bars) increased the duration of the step cycle ($p<.05$) for a greater period of time than stimulation of the ipsilateral LGS nerve (containing the chronically implanted LGS cuff) during decerebrate walking. *(i MG ipsilateral medial gastrocnemius, i St ipsilateral semitendinosis, i VL ipsilateral vastus lateralis).*

the intact and decerebrate preparations in 3 animals by first stimulating the LGS nerve at group I strengths while the animals were intact and later after they had been decerebrated. The time between recording sessions was 3-4 hours and the same stimulating and recording cuffs were used. In all 3 animals, stimulation of the LGS group I afferents had a greater influence on the duration of stance during spontaneous decerebrate walking (figure 3-8). The effects were also less variable during decerebrate stepping and in 1 of the 3 animals the stimulation of the LGS group I afferents consistently prolonged the extensor burst for the duration of the stimulus train (see figure 3-4B). To estimate the extent to which chronic axotomy affected the ability of LGS group I afferent stimulation to prolong stance, we compared the stimulation of the previously implanted LGS with that of a freshly implanted LGS cuff on the other hindlimb in 2 of the 3 decerebrate cats. In both of these animals, stimulation of the previously implanted LGS nerve was less effective in prolonging stance than equivalent stimulation of the contralateral LGS nerve. These results are consistent with those presented in chapter 4 and 5 which showed that a reduction in the effectiveness of LGS stimulation can take place within 3 days of LGS nerve axotomy.

In all 3 of the animals that were decerebrated the magnitude of the MG EMG significantly increased during LGS nerve stimulation ($p<.05$). In 2 of these 3 animals, both the long latency (35-60 ms) and the short latency (monosynaptic or possibly disynaptic) components (7-20 ms) of the EMG burst following LGS stimulation were increased (figure 3-4B). In the remaining animal, only the long latency component was significantly increased. The amplitude of the long latency component recorded in the MG EMG increased by 30-70% ($p<.05$) following decerebration. Stimulation of the LGS group I afferents did not increase the amplitude of the VL EMG at latencies less than 60 ms in any of the 3 decerebrate animals. However, LGS stimulation did increase the amplitude of the VL extensor burst during late stance, compared to unstimulated trials (figure 3-4C).

Variation in influence of LGS group I afferents in decerebrate animals

In the course of our experiments on walking decerebrate animals we made some previously unpublished observations that may be related to the variation in the influence of LGS group I afferents in intact cats. Although we typically worked with spontaneously walking decerebrate cats, in 4 animals we used MLR stimulation to evoke stepping when they did not step spontaneously. In these animals we were able to control the vigor of walking by simply increasing either the frequency or the voltage of the MLR stimulus. We found that the degree to which extensor group I afferents prolonged the extensor burst was inversely correlated to the intensity of the MLR stimulus (figure 3-9). At high MLR stimulus strengths, the effectiveness of group I stimulation modestly affected the duration of the extensor burst, whereas at lower intensities similar group I stimulus trains could powerfully affect the duration (3 out of 4 cats). These results suggest that increased locomotor activity induced by supraspinal locomotor centres can modulate the effectiveness of the group I oligosynaptic pathway. An additional observation consistent with these findings was that during bouts of vigorous stepping, extensor group I effectiveness was frequently reduced in spontaneously walking decerebrate cats. We also noticed that the effectiveness of extensor group I stimulation sometimes increased as the walking pattern became progressively weaker.

Discussion

The main result of this study is that stimulation of group I extensor afferents can functionally prolong the stance phase and delay the onset of flexion in the intact walking cat. Although these results were qualitatively similar to previous investigations that have used acute cat preparations, there were a number of important differences. The effects on the duration and amplitude of the extensor bursts were not as large as results obtained using decerebrate walking cats (chapter 2) and those of others who have used reduced preparations (Guertin et al., 1995; Conway et al.,

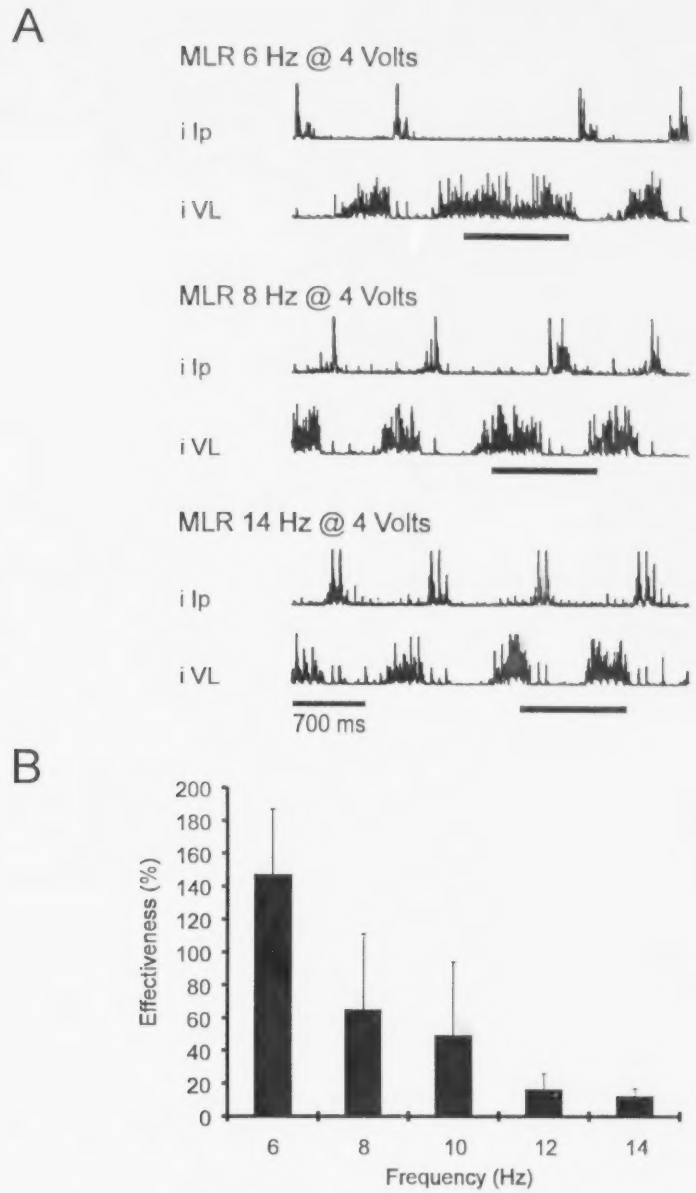

Figure 3-9: Increasing the MLR stimulus strength decreases the effectiveness of stimulation of extensor group I afferents in prolonging stance. A. Rectified and filtered EMG traces showing the effects of increasing the MLR frequency on the influence of stimulation of the group I afferents in the MG nerve (trains: 1000 ms duration, 1.8 x T, 200 Hz, 200 ms delay triggered off the i Vl burst). The dark lines show when the stimulus train was on. Note that at a constant MLR stimulation level of 6 Hz, the stimulus train prolonged the duration of stance and delayed the i Ip burst for the duration of the stimulus train. When the frequency of MLR stimulation was increased to 14 Hz the effectiveness of MG stimulation decreased. B. Bar chart showing the average effectiveness of MG stimulation during MLR stimulation at various frequencies. The error bars reflect the standard deviation. All trials are from one cat. *(i Ip ipsilateral iliopsoas, i VL ipsilateral vastus lateralis).*

1987; Pearson et al., 1992). In all the intact animals, stimulation of the LGS group I afferents rarely prolonged stance for the duration of the stimulus train (e.g. figure 3-0B). Another difference was observed when extensor group I afferents were stimulated during early flexion. Instead of inhibiting the flexor burst and resetting the rhythm to extension as has been reported in reduced preparations (chapter 2; Conway et al., 1987; Guertin et al., 1995a) the initiation of the extensor burst was advanced with little effect on the amplitude of the flexor burst. Finally, stimulation of the group I extensor afferents produced variable effects on the duration of stance. For example, stimulus trains could prolong stance for the duration of the train in some trials, yet have a modest effect in the very next trial.

Effects on the amplitude of the extensor burst

An important aspect of our experiments was that stimulation of the LGS group I afferents had an effect on the kinematics of the step cycle that were related to increases in the duration and amplitude of the EMGs. This was evident in animals which had a pronounced yield of the ankle during unperturbed walking after axotomy of the LGS nerve (also see chapter 5 - figure 5-6). In these animals stimulation of the LGS group I afferents could abolish the yield of the ankle and increase the amplitude of the MG EMG burst. This was an especially important result because it demonstrated that stimulation of heteronymous group I extensor afferents could contribute to the functional recovery of animals. Whether they actually do so is a subject of a later chapter (chapter 5). Somewhat puzzling is that stimulation of LGS group I afferents decreased the yield around the knee despite the fact that the VL EMG did not increase. One possible explanation is based on the biomechanics of the limb. Since MG is a biarticular muscle which extends the ankle and flexes the knee, an increased yield of the ankle during stance would be expected to increase the flexor torque around the knee joint (Van Ingen Schenau, 1989). The additional excitation of

the MG muscle produced by stimulation of the LGS nerve would tend to reduce the yield of the ankle and would thus indirectly reduce the flexor torque around the knee.

While the kinematics show that stimulation of group I extensor afferents can correct a deficit in gait following axotomy of the LGS nerve it is not clear which reflex pathways mediate these effects. At present we know of three excitatory group I extensor pathways that are open during locomotion and could increase the amplitude of the MG EMG (Pearson, 1995). These pathways from extensor group I afferents onto the extensor motoneuronal pool include: (1) an oligosynaptic pathway that has been postulated to project onto the extensor half-centre and would be expected to generally excite the extensor motoneurons (Gossard et al., 1994), (2) a recently discovered disynaptic excitatory pathway that also produces an generalized excitation onto extensor motoneurons (Angel et al., 1996) and finally (3) the well known monosynaptic pathway from group Ia afferents which projects in a more localized fashion to various extensor motoneuronal pools (Eccles et al., 1957). While the relative contributions from each of the three pathways to the increase in the amplitude of MG is beyond the scope of the experiments in this chapter it is likely that the oligosynaptic pathway does contribute. This is demonstrated by the fact that simultaneous stimulation of PL and the LGS group I afferents could greatly increase (figure 3-5) the amplitude of the MG EMG. Since group I afferents from PL mainly project polysynaptically onto the MG motoneuronal pool, this increase in amplitude represents additional excitation from the oligosynaptic or disynaptic pathway. This type of summation has been observed before in decerebrate walking cats (chapter 2; appendix 2). The possibility that these actions are mediated by parallel or common set of interneurons awaits future investigation. Another clue that oligosynaptic group I extensor pathways contribute to the increase in the amplitude of the extensor burst is that the amplitude of the MG EMG following LGS group I stimulation had two distinct components. The long-latency component in the MG EMG occurred

at a latency of 35-50 ms which is similar to that reported by Pearson and Collins (1993) when they stimulated the PL group I afferents and recorded large amplitude increases in the MG EMG using clonidine treated spinal cats. These effects reported by Pearson and Collins (1993) likely represent pure excitation from the oligosynaptic pathway which accesses the half centre since: (1) there are few monosynaptic connections from PL to MG (Eccles et al., 1957) and (2) the disynaptic pathway is closed in low spinal cats (McCrea et al., 1995a). It is likely that the inputs from the monosynaptic, disynaptic and oligosynaptic pathways onto the extensor motoneuronal pool combine to produce the localized excitation of the MG extensor burst (which was not observed in the VL EMG) by stimulation of the LGS group I afferents. This pattern of localized excitation of ankle extensors from stimulation of ankle group I afferents has also been observed in the fictively locomoting decerebrate cat (Guertin et al., 1995). Intrinsic membrane properties of the extensor motoneurons may also contribute to the localized increase in amplitude (Brownstone et al., 1994). For example it is known that extensor group I stimulation can produce larger slow EPSPs when the extensor motoneurons are depolarized (Brownstone et al., 1994). Thus, when a stimulus train activates the LGS group I afferents, monosynaptic excitation could depolarize the MG motoneuronal pool to a level at which the longer latency effects from the oligosynaptic pathway would become more effective.

The weaker effects of stimulating the axotomized LGS group I afferents in intact animals were partly due to plasticity of the LGS group I pathway.

In the animals that we from on consecutive days there was a progressive reduction in the effects of stimulating the LGS group I afferents on prolonging the extensor burst (figure 3-7). To test whether the reduction in effectiveness could be entirely attributed to axotomy of the nerve we decided to decerebrate some of the intact cats in which we had previously recorded data. If axotomy of the nerve were the sole reason for the reduced effects in the intact cat, we would

expect that stimulation of the extensor group I afferents would have similar effects in both preparations. This was not the case. The effects of LGS group I stimulation were greater in the decerebrate state, suggesting that part of the difference must be dependent on the type of preparation used. However, although stimulation of the LGS group I afferents prolonged stance more robustly than in the intact state, the effects were still less than stimulation of an acutely cut LGS nerve in the control leg. In decerebrate walking animals in which the LGS nerve had been axotomized before the acute procedure for 3-28 days, the effects of stimulating the nerve were also reduced 3 days after axotomy (chapter 4 & 5). These findings suggest that in the intact animal we are underestimating the effects of stimulating the group I afferents. However, even in an animal in which we recorded data as early as 8 hours after surgery, the effects were still not as great as those observed in the decerebrate cat, suggesting that true differences exist. It is not likely that plasticity occurs within 8 hours since: (1) decerebrate walking cats produce results for over 16 hours without a diminution in the effects of stimulating LGS group I afferents and (2) the results presented in chapter 4 and 5 show that this plasticity tends to develop over days not hours (see also figure 3-7).

Stimulation of group I extensor afferents had variable effects on the duration of the extensor burst

Stimulation of extensor group I afferents produced variable effects on the duration of stance in the intact cat. Several factors were identified that contributed to this variability. Firstly, the effects of stimulating the group I extensor afferents showed a tendency to habituate after repeated trials. This could either occur rapidly in one trial (e.g. figure 3-0C) or over the course of a recording session. One possibility that may explain this rapid decline is that synchronous activation of all the group I afferents in one nerve may be recognized as an artificial stimulus by the animal. In this regard, Johannsson and Westling (1987) have demonstrated that cutaneous

afferents can signal the degree of precision grip needed in humans. However subjects rapidly adapted to the electrical stimulation of cutaneous afferents, yet never adapted to normal tactile stimuli. The animal may also learn to decrease the transmission in the stimulated nerve since the effects of group I extensor stimuli tended to destabilize the gait of the animal.

Another cause of the variability may have been due to changes in the intensity of the descending drive from supraspinal centres. In support of this idea we found that the effectiveness of extensor group I stimulation could be decreased in decerebrate animals by increasing the intensity of the MLR stimulus (figure 3-9). Recently, Noga et al. (1995) have found that the group I and II field potentials produced in the intermediate zone of the spinal cord are reduced by over 55% of control values when a conditioning train of stimuli is applied to the cuneiform nucleus (thought to form part of the MLR (Jordan, 1991)). If the intensity of the MLR stimulus was decreased, there was less inhibition of the group I and II field potentials. Furthermore, stimulation of the raphe nucleus, the locus coeruleus, and the red nucleus have all been shown to depress field potentials produced by group I and II afferents (Noga et al, 1992; Jankowska et al., 1993; see also Hongo et al., 1969; 1972). This suggests that descending tracts active during locomotion produce a generalized tonic depressive effect on group I transmission. In light of this it is interesting that decerebration of the intact animals led to an increase in the effectiveness of stimulating the LGS group I afferents in all the animals tested. It is well known that decerebration of cats can increase the excitability of some reflex pathways and decrease others (Baldissera et al., 1981; Eccles and Lundberg, 1958; Kuno and Perl, 1960; Hoffer et al., 1990). Furthermore lesions of the cortex in intact animals cause the release of excitatory group I extensor pathways which suggests the cortex normally exerts an inhibitory influence on the expression of these pathways (Pacheco and Guzman-Flores, 1969). Taken together these findings

suggest that the oligosynaptic group I pathway is highly modulated by descending inputs. In accord with this idea, we noticed in one intact animal that if we increased the speed of the treadmill the effects of stimulating the extensor group I afferents on the duration of the step cycle decreased (unpublished observations; appendix 2).

The effects of stimulating extensor group I afferents depended on the locomotor task

A consistent observation was that the effects of stimulating the LGS group I afferent were greater during periods of bipedal than quadrupedal walking. These observations suggest that during bipedal walking there is a greater reliance on group I extensor input to control the duration of the extensor burst. Interestingly, Goldberger, (1977) arrived at a similar conclusion when he examined the recovery of cats with a unilateral deafferentation. While the animals could regain the ability to quadrupedally walk using the affected limb, they could not bipedally walk. If one dorsal root was spared during the deafferentation the animals always regained the ability to bipedally walk. How could this increased reliance on sensory input occur? Firstly, activity of the forelimbs may decrease the effectiveness of extensor group I feedback. In support of this we observed in the decerebrate cat that when all 4 limbs were stepping, stimulation of the group I extensor afferents was generally less effective than when the forelimbs were stationary (unpublished observations; appendix 2). We know from studies in intact animals that movement of the forelimbs can affect the timing of the hindlimbs (Cruse and Wernicke, 1992), suggesting that either propriospinal input from the forelimbs or input from the brainstem onto both the fore and hindlimb may be acting to coordinate the rhythm produced in both girdles (Cruse and Wernicke, 1992; Bem et al., 1994). Since a separate pattern generator exists for each limb and these generators are linked it is likely that a fixed sensory input from extensor group I afferents will have a reduced effect when all limbs are active.

A second possibility is that the effectiveness of group I stimulation during walking is state dependent. Task-dependent modulation of afferent pathways have been described in humans and many other vertebrates and invertebrates (Pearson, 1993; Prochazka, 1989). This modulation ensures that the modulatory effects of afferent input are optimized to the ongoing locomotor behavior. For example, while locomoting across uneven ground, cats must continually modify their gait to compensate for observed irregularities in their environment (Drew, 1991). The motor cortex likely plays a key role in this gait modification by integrating information from various sensory modalities, especially from the visual system (Drew, 1991). It is likely that these modifications of the gait pattern are accomplished by cortical access access to the basic rhythm generating circuitry of the spinal cord (Drew, 1991). It would be likely that the ability of proprioceptive feedback to control rhythm generating centres would be changed by cortical input to the cord. A more likely scenario is that these modifications of the rhythm generator change the strength of reflex pathways that can affect the locomotor rhythm. Since cells within the motor cortex fire at a higher rate as the complexity of the locomotor task increases (Beloozerova et al., 1993) it is interesting to speculate on whether these changes in the firing rate would affect the modulation of the group I extensor pathway discussed in this paper. A possibility is that during quadrupedal walking the inputs from extensor group I afferents were gated by the motor cortex to a greater degree than during bipedal walking possibly by presynaptic inhibition of the afferents (Equibar et al., 1994). We suspect that bipedal walking is a simpler task than quadrupedal walking and certainly does not require continuous visual input for its execution (cats regularly ate from a bowl of food without looking up). It would be interesting to see if the effects of stimulating the group I extensor afferents were increased during quadrupedal locomotion if cats were denied visual information.

The extensor group I pathway is not the only pathway that can control the stance to swing transition. Activation of flexor group I and/or II afferents can inhibit stance and promote an earlier onset of flexion in the spontaneously walking cat preparation (Hiebert et al., 1996). Thus, they have the opposite effect on the duration of stance than extensor group I afferents. It is interesting to speculate whether increased cortical activity may shift the weighting from the extensor group I afferents to the flexor group I afferents. Since microstimulation of the motor cortex can also inhibit the stance phase (Drew, 1991), augmentation of the response from flexor group I and II afferents may be one way in which this could be accomplished.

Functional relevance of the group I extensor pathways in intact cats

The ability of extensor group I afferents to affect the duration and the amplitude of the extensor burst in intact cats suggests these sensory signals are normally involved in controlling the stance to swing transition in the cat. Moreover this is a conservation conclusion since we know that we were underestimating the import of group I extensor input in this investigation. The functional consequences of extensor group I activity probably differ depending on the ensemble activity from extensor muscles. If many extensor muscles are active simultaneously, the effects on the amplitude and duration of the extensor burst would be expected to be quite large. However, if only extensor muscles around a certain joint are activated, a more localized response would be expected and the timing of the limb would be weakly affected. Moreover our results suggest that the input from the extensor group I afferents are flexibly adjusted by descending inputs. Thus, in this study we have had the opportunity to observe the task-dependent modulation of *functionally* relevant extensor group I pathways. Thus, this study represents a first step in understanding how extensor group I input is incorporated into the ongoing behavior of mammals.

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Chapter 4

Plasticity of the extensor group I pathway controlling the stance to swing transition in the cat[4]

Introduction

Plasticity of reflex pathways can occur in response to muscle inactivity, axotomy of peripheral nerves, conduction block of afferent activity, and operant conditioning (Wolpaw and Carp 1993; Mendell 1984). Eccles and colleagues were among the first to show that cutting a peripheral nerve results in diminution of the monosynaptic reflex from group Ia afferents to extensor motoneurons (Eccles et al. 1959; cf. Eccles and McIntyre 1953). This decrease occurs after 2 weeks of the cut and reaches 50% of control values within 3 weeks. On the other hand, a study by Gallego et al. (1979) and recently confirmed by Webb and Cope (1992) has shown that the amplitude of the group Ia excitatory postsynaptic potentials (EPSPs) is increased over a period of 2 weeks after the medial gastrocnemius (MG) nerve is deactivated by tetrodotoxin (TTX). This suggests that axotomy and not disuse is a causal factor in the reduction of synaptic efficacy after nerve section. Some investigators have also shown an increase in the synaptic efficacy of the homonymous group Ia pathway to MG muscles after axotomy of nerves to synergists (Eccles et al. 1962). However, this result was not confirmed by Walsh et al. (1978) who failed to report any significant increase in the MG Ia EPSP after axotomy of the lateral gastrocnemius and soleus (LGS) nerves. Plasticity can also occur in the monosynaptic group Ia pathway without peripheral

[4] A version of this chapter has been published. Whelan, P. J., Hiebert, G. W., and Pearson, K. G. Plasticity of the extensor group I pathway controlling the transition from stance to swing in the cat. J. Neurophysiol. 74: 2782-2787, 1995. As primary author of this paper I was heavily involved in the performance, planning and execution of the experiments and I performed all of the subsequent data analysis. I wrote the initial draft of this document, which was edited by Dr K.G. Pearson.

nerve lesions. In the monkey, Wolpaw (1987) reported that the magnitude of the monosynaptic reflex can be operantly conditioned to either increase or decrease. Since the reflex remains conditioned after spinalization, its locus must lie within the spinal cord, although recent evidence suggests that supraspinal control of the plasticity also occurs (Wolpaw and Carp, 1993). The general picture to emerge from all these studies is that there are many ways to alter the transmission in the monosynaptic pathway but it usually takes weeks or even months for this plasticity to develop.

In the present study we have examined plasticity in the reflex pathway regulating the transition from stance to swing in walking decerebrate cats (Chapter 2). This pathway has been described only relatively recently, and it consists of an oligosynaptic excitatory pathway from extensor group I afferents to extensor motoneurons that is open only during locomotor activity or under conditions that promote locomotor activity such as the administration of L-DOPA (Gossard et al. 1994; McCrea et al. 1995; Pearson and Collins 1994). This pathway probably includes elements of the rhythm generating network since stimuli that evoke the oligosynaptic EPSPs also reset and entrain the locomotor rhythm (Conway et al. 1987; Pearson et al. 1992).

Previously we reported that in decerebrate walking cats stimulation of the group I afferents in extensor nerves during stance caused an increase in the duration of the extension burst and also delayed the onset of the flexion (Chapter 2). Stimulation of the group I afferents in the LGS nerve had a particularly powerful effect usually prolonging the extensor burst for the duration of the stimulus train. In contrast stimulation of other extensor nerves did not prolong the extensor burst for the duration of the train. In preliminary experiments on chronic intact cats in which the LGS nerve had been cut for some days we noticed that the effectiveness of LGS nerve stimulation was reduced compared to the effects we had observed in decerebrate cats. This

prompted us to ask whether the decrease in efficacy was due to axotomy and if it was, whether there was an associated increase for the non-denervated synergist MG. To answer these questions we have cut the LGS nerve of a hind leg in a series of cats and at various times after this operation compared the effects on stance duration of stimulating each of the LGS and MG nerves in both hind legs. The contralateral leg acted as a control for judging any changes in reflex efficacy in the leg containing the previously transected LGS nerve.

Methods

All animals used in this study were cared for in accordance with the guidelines published by the American Physiological Society and the experimental procedures were approved by the University of Alberta animal welfare committee. Experiments were carried out in 11 adult cats of both sexes. Under Halothane anesthesia and aseptic conditions the nerve supplying the LGS was exposed and transected close to the muscle. The proximal nerve was tied with 6.0 silk to allow for subsequent detection. An antibiotic (Ayercillin, 1 cc) and if necessary an analgesic (Buprenorphine; .005 - .01 mg/kg) were administered for one week after surgery. Animals were allowed out of their cages on a daily basis after surgery and the cages were large enough (dimensions: 73 cm wide by 69 cm deep by 84 cm high) to permit the animal to move and to jump onto a ledge. No attempt was made to actively exercise them. After a period of 3 to 28 days, the acute surgical and experimental procedures were performed on the cats. Only an abridged description of the acute procedure is included here, for a full description please see chapter 2. Halothane anesthesia was administered to each animal during acute surgery. In initial experiments, 3 cats had their chronically cut left LGS, and acutely cut left plantaris (PL), right LGS and right PL nerves tied into the stimulating cuffs. In 7 cats the chronically cut left LGS nerve and the acutely cut left MG, right LGS and right MG nerves were tied into the stimulating cuffs. In 1 cat only the left and right MG nerves were cuffed. The stimulating cuffs were

manufactured from latex and were each about 6 mm long with an inside diameter of about 3 mm. A silicone recording cuff was placed around each of the sciatic nerves to record the strength of the stimulus. The threshold of the electrical stimulus to the extensor nerves was taken as the minimum voltage necessary to produce a visually detectable sciatic potential. The strength of the stimulus was expressed in multiples of this threshold level. Bipolar stainless steel recording electrodes (Cooner Wire Company, AS632) were sewn into the following muscles of both hindlegs to record electromyographic (EMG) activity during walking: MG (in the 3 cats which did not have the MG nerve cut), vastus lateralis (VL), semitendinosus (St) and iliopsoas (IP). The wires from both the stimulating and the EMG electrodes were led subcutaneously to a multipolar connector on the back of the cat. After finishing this procedure the cats were placed over a motorized treadmill and then decerebrated. The Halothane anesthesia was discontinued at this time. The cats were supported under the abdomen by a sling to maintain lateral stability and to aid in weight support. In 10 cats spontaneous bouts of walking occurred in response to a moving treadmill. Occasionally manual stimulation of the perineum was used to evoke these bouts of locomotion. Although the triceps surae were denervated most cats produced a stepping pattern that was kinematically equivalent to that produced by normal decerebrate cats. One cat did not step at all. The speed of the treadmill was set between 0.25 - 0.35 m/s depending on the animals step pattern. During stance a stimulus train to the appropriate extensor nerve (1.8 - 2 x T) was triggered 200 ms after the onset of the extensor EMG (mostly VL; in 3 initial experiments MG). The duration of the stimulus train was 1000 ms, which was short enough to allow stimulation of a normal LGS nerve to prolong the extensor burst for the duration of the train yet long enough for stimulation of a normal MG nerve to not prolong stance for the duration of this train. This was important because it allowed for reductions in LGS efficacy *and* increases in MG efficacy to be observed in the experimental limb.

All data were recorded using a Vetter 4000A PCM recorder. Later, selected sequences were stored on computer disc using the Axotape (Axon Instruments) data acquisition system installed on a Microexpress 486 computer. Data analyses were carried out using custom programs that could retrieve data from the axotape files. The cycle periods before, during, and after the stimulus were calculated only during regular sequences of walking. Each cycle period was calculated as the time between the occurrence of successive St or Ip bursts. A spreadsheet program (Microsoft Excel 5.0) was used to calculate the mean and standard deviation for these cycle periods and Student's t-tests were administered to detect significant differences between the conditions. The data were normalized according to the equation below to allow for comparisons between cats and between control and experimental nerves.

Equation 1:

Percentage effectiveness = (b-a)/(c-a) x 100

Where *b* equals the stimulated cycle period, *a* represents the control cycle period and *c* represents the time from the first flexor burst before the stimulated extensor burst to the offset of the stimulus train (see figure 4-0B for an illustration of what the variables measured). The percentage effectiveness is a measure of how powerfully the stimulus could affect the step cycle. For example if the stimulus was 100% effective the next flexor burst would be held off until the end of the stimulus train. By contrast if the percentage effectiveness was 0% the stimulus would have no effect on the cycle period.

In three cats, the MG nerve was left intact and this presented us with the opportunity to test whether the cut LGS afferents were still conducting normally to the spinal cord. Heteronymous H-reflexes in the MG muscle were elicited by stimulating the cut LGS nerve at group I strengths (0.2ms duration, single pulses). In two additional cats we examined whether axotomy of the LGS

nerve caused alteration in conduction properties to the group I extensor afferents by recording intracellularly from LGS motoneurones 7 and 17 days after axotomy. The cells were identified by antidromically activating them by stimulation of the LGS nerve. After a cell was identified threshold was established for LGS nerve stimulation as described above. The stimulus was increased gradually to 1.5 x T and the resultant average post-synaptic potential (PSP) was recorded using custom software. For a full description of the methods used in the intracellular experiments see McCrea et al. (1995).

Results

The objective of the experimental procedure was to compare the effectiveness of stimulating either the LGS or MG nerve in the experimental leg (LGS nerve cut from 3-28 days earlier) with the effectiveness of stimulating the same nerve (with the same stimulus parameters) in the control leg. Plasticity was judged to occur if there was a significant difference between the experimental and control legs *in the same animal*. In 7 out of 10 cats we compared the effects of stimulating the MG nerves, and in 9 out of 10 cats we compared the effects of stimulating the LGS nerves (both measurements were made in 6 cats). All but one of the experimental cats walked spontaneously after decerebration.

The efficacy of MG nerve stimulation was increased compared to the control hindlimb

Stimulation of the group I afferents in the MG nerve of the control leg resulted in a modest increase in the extensor burst duration and a corresponding delay in time of the onset of the next flexor burst as shown in figure 4-0A. The shaded bars in figure 4-0C summarize the results from stimulation of the control MG in all the cats studied. Stimulation of control MG was usually only 20-30% effective in delaying the flexor burst and extending stance. In only 1 out of 7 cats was the control MG response greater than 50%. By contrast stimulation of the group I afferents from the MG nerve in the experimental leg usually had a strong effect as shown in figure 4-0B. The

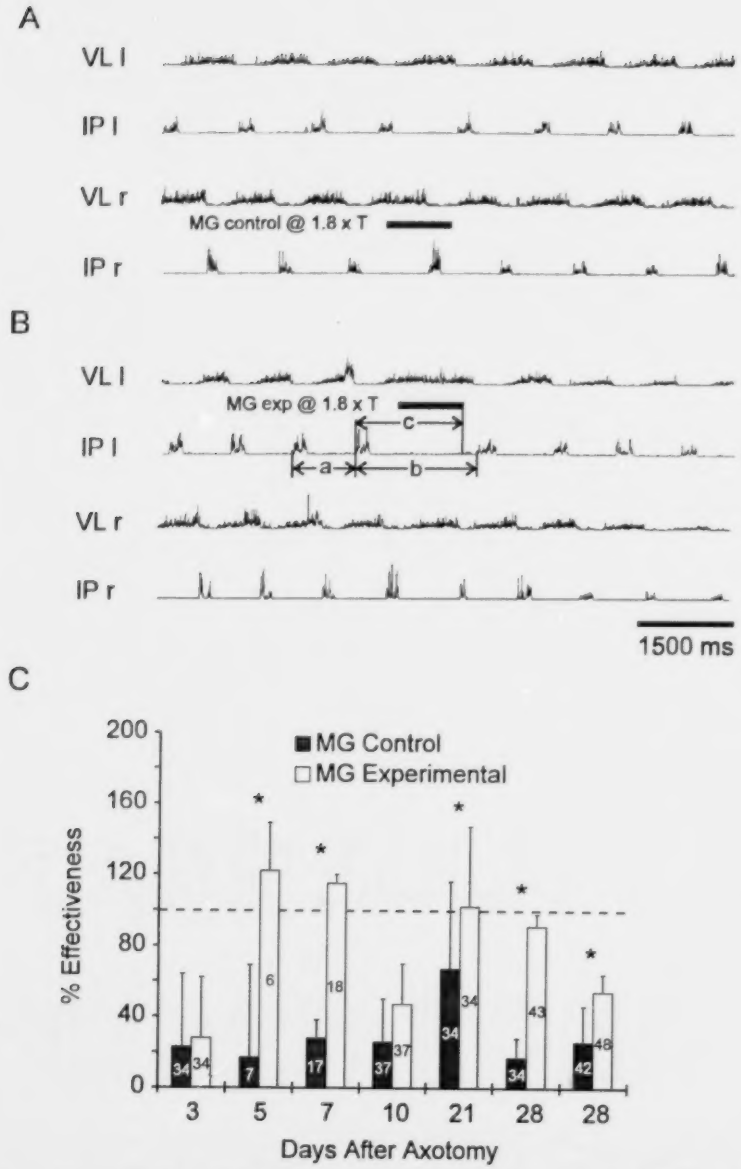

Figure 4-0: Axotomy of the LGS nerve increases the ability of group I afferents in the MG nerve to prolong extensor burst activity. A,B Rectified and filtered EMG from the hindleg muscles showing individual trials in the control (A) and experimental (B) legs (stimulus trains: 1000 ms duration, 200 Hz, 1.8 x T). Note that in the control leg (A) stimulation of the MG modestly increases the duration of the extensor burst. In contrast after axotomy of the LGS nerve for 21 days, stimulation of the MG nerve using similar parameters results in a relatively large increase in the duration of the extensor burst. The percentage effectiveness of the stimulus train = (b-a)/(c-a) x 100 (see methods for further details). C. Bar graph showing the mean percent effectiveness of MG stimulation of the control (shaded bar) and experimental (open bar) legs for each individual experiment. The error bars represent the standard deviation. The * indicates that there is a significant difference between the two conditions ($p<.05$). The numbers within each bar illustrate the number of trials that were used to calculate the average. The number on the abscissa below each bar indicates the duration of axotomy. (*VL l* vastus lateralis - left leg, *IP l* iliopsoas - left leg, *VL r* vastus lateralis - right leg, *IP r* iliopsoas - right leg, *MG* medial gastrocnemius).

data summarized in figure 4-0C shows that in 4 out of 7 cats the stimulus train to the experimental MG nerve effectively held off flexion and prolonged the extensor burst for the duration of the stimulus train (> 100% percentage effectiveness). In 5 out of 7 cats there was a significant increase in the efficacy of the experimental MG nerve after stimulation at group I strengths compared to stimulation of the control MG at similar strengths.

figure 4-0C also shows that there was no tendency for the efficacy to increase progressively over the period from 5 - 28 days after LGS axotomy. Despite the considerable variability from cat to cat it was clear that substantial increases could occur within one week. In two cats that had their LGS nerves axotomized for 5 and 7 days respectively, a large asymmetry was observed between the effects of MG stimulation in control and previously axotomized limbs.

The differences between the effects of stimulating the MG nerve in experimental and control legs were generally visually apparent. In the experimental leg stimulation of the MG prolonged stance by producing a generalized extension of the entire leg. In comparison, the control MG generally had a small effect on the kinematics of the step cycle (not shown).

The efficacy of the axotomized LGS nerve was reduced compared to the control hindlimb.

As reported in chapter 2, stimulation of group I afferents in the LGS nerve of the control leg resulted in a large increase in the stance phase duration with the stimulus usually prolonging the extensor burst for the duration of the stimulus train as shown in figure 4-1A. Note that in this example the onset of the ipsilateral flexor activity occurred after the stimulus offset. The contralateral rhythm was generally unaffected by the stimulus except in cases when the stimulus offset occurred immediately before the onset of contralateral flexor activity in which case the contralateral flexor burst would be delayed until after the ipsilateral flexor burst had been completed (data not shown). Data from all the experiments is summarized in figure 4-1C.

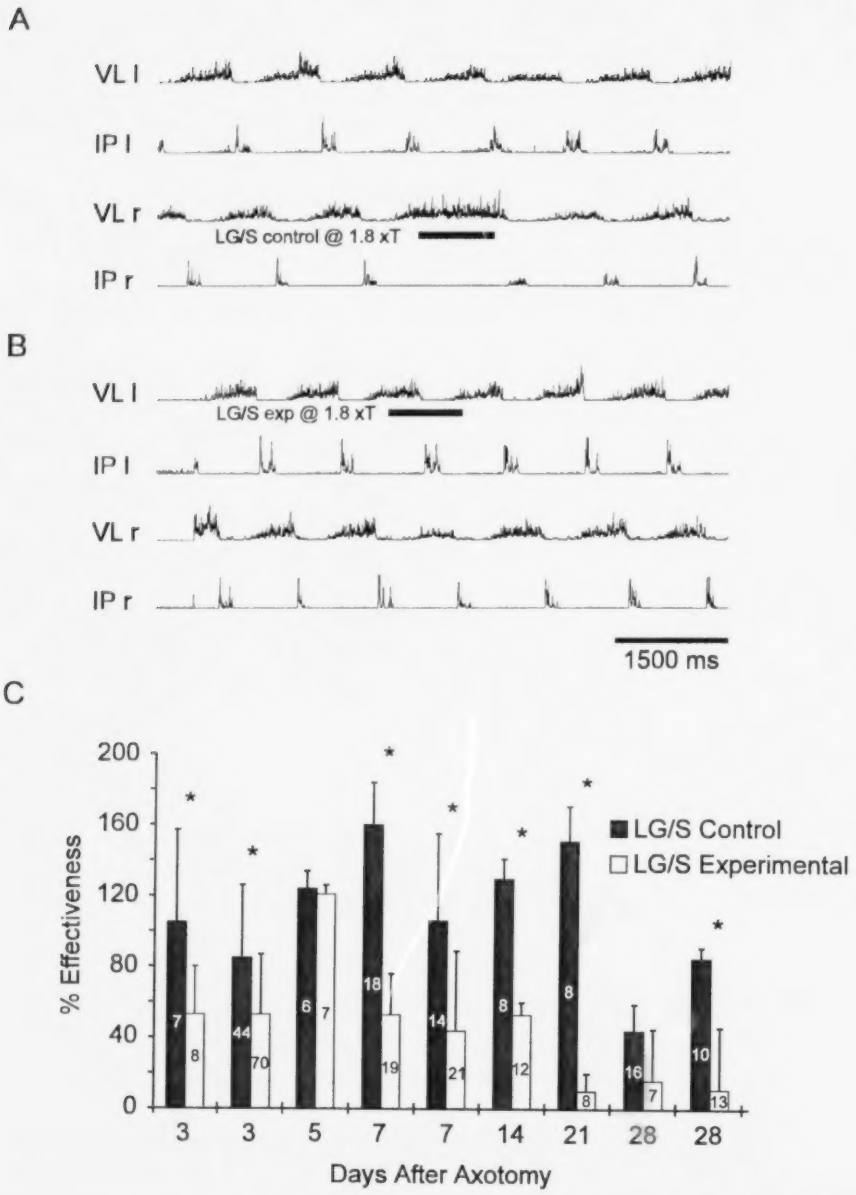

Figure 4-1: Axotomy of the LG/S nerve reduces the ability of group I afferents in the LG/S nerve to prolong extensor burst activity. A,B Rectified and filtered EMG from hindlimb muscles showing individual trials in the control (A) and experimental (B) legs (stimulus trains: 1000 ms duration, 200 Hz, 1.8 x T). Note that in the control leg (A) stimulation of the LG/S nerve produced a large increase in the duration of the extensor activity for the duration of the stimulus train. In contrast in the experimental leg (B) after axotomy of the LG/S nerve for 21 days, stimulation of the nerve using similar parameters only modestly increased the extensor burst. C. Bar graph showing the mean percent effectiveness of LG/S stimulation of the control (shaded bar) and experimental (open bar) legs for each individual experiment. The error bars represent the standard deviation. The * indicates that there is a significant difference between the two conditions ($p<.05$). The numbers within each bar illustrate the number of trials that were used to calculate the average. The numbers on the abscissa under each bar indicate the duration of the axotomy. (*LG/S* lateral gastrocnemius and soleus)

Stimulation of the control LGS nerve effectively prolonged stance in 8 out of 9 cats. In only 1 cat was the stimulus to the control LGS nerve less than 80% effective in holding off the flexor burst. In contrast, stimulation of the group I afferents in the previously cut LGS nerve had a relatively modest effect on the duration of the extensor burst (figure 4-1B and 4-1C). In 7 out of 9 cats there was a significant reduction ($p< 0.05$) in the efficacy of the axotomized LGS nerve after stimulation at group I strengths compared to stimulation of the control LGS nerve at similar stimulus strengths while in 5 out of 9 cats there was a greater than 40% difference in the percentage effectiveness of the LGS nerve stimulation between the experimental and control hindlimb. Only two animals (day 5 and one day 28) showed no evidence of asymmetry. Similar to the results for MG the reduction in the efficacy of the LGS nerve took place rapidly. Within 3 days of axotomy clear differences could be observed between stimulation of the control and experimental LGS nerves, although no overall trends with time could be observed (figure 4-1C).

One possible reason for the reduction in LGS efficacy was that axotomy of the LGS nerve could have caused a reduction in the diameter of the large group I afferents. Electrical stimulation preferentially recruits group I afferents at low stimulus strengths due to their large axonal diameter. If the diameters of group I afferents were reduced relative to the α-motoneurons a normal sciatic nerve potential could be produced even though fewer group I afferents would be activated. Two sets of data indicate that this did not occur. In two cats we were able to elicit heteronymous H-reflexes in the MG muscle by stimulation of the previously cut LGS nerve. The H-reflex is the electrical equivalent of the tendon tap reflex and is used to infer the transmission of monosynaptic group Ia afferents to the α-motoneurons. The first sign of an H-reflex occurred at low strengths (1.2 x T) and reached a maximum amplitude at 1.8 x T, indicating Ia transmission at low stimulus strengths. Figure 4-2A shows the averaged response in the H-reflex in MG EMG evoked after stimulation of the cut LGS nerve at 1.8 x T. Second, in two other cats

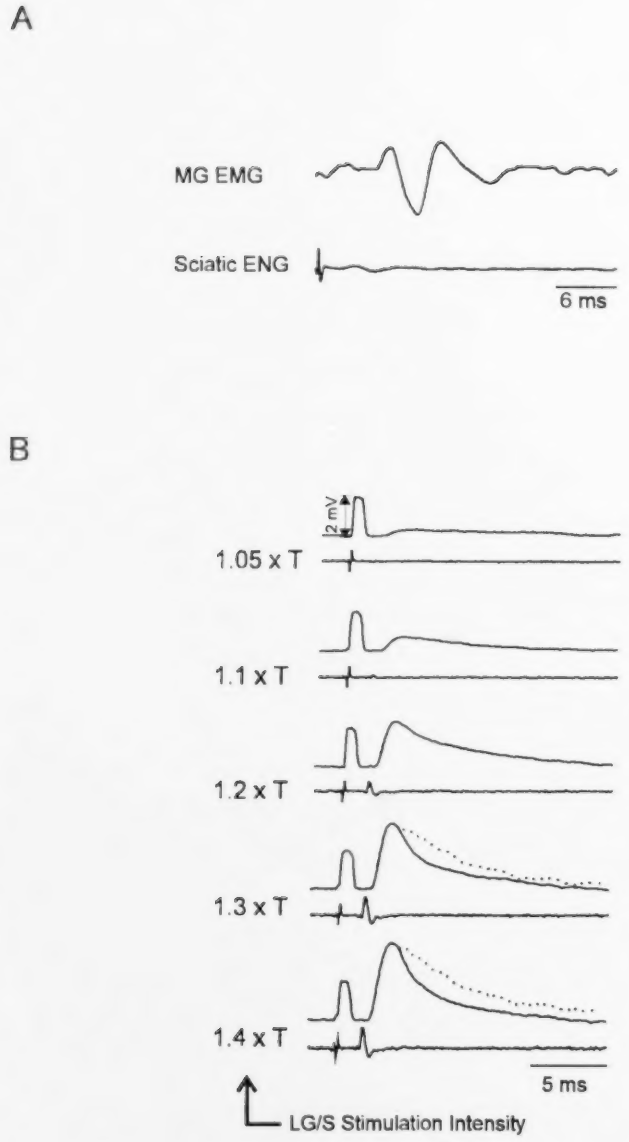

Figure 4-2. A. Heteronymous H-reflex from MG muscle in response to stimulation of the LG/S nerve that had been cut for 14 days (single pulses: 1.6 x T: .2 ms duration). The top trace is the average (n=32) EMG from the MG muscle while the bottom trace indicates the sciatic nerve potential. The cat was in a quiescent state with an tonic background level of EMG. B: Intracellular recording from a LG/S motoneuron show a normal recruitment of PSP's in response to stimulation (average is between 15 to 20 depending on the trace) of an axotomized LG/S nerve (17 days after axotomy). Note that at 1.3 x T the falling edge of the EPSP is attenuated indicating that disynaptic inhibition from Ib afferents is present. The top trace of each set indicates the averaged membrane potential from a LG/S motoneuron. The dotted lines present on the traces with stimulation of 1.3 x T & 1.4 x T indicate the expected EPSP if no Ib inhibition had been present. The expected EPSP was obtained by amplifying the EPSP from 1.1 x T. The bottom trace indicates the cord dorsum potential.

we recorded homonymous PSPs from LGS motoneurons in response to LGS stimulation after the nerve had been axotomized for 7 and 14 days. In both cases the group I profile of the synaptic potentials was normal (figure 4-2B). The monosynaptic EPSP was evoked at the lowest threshold and stimulation at levels greater than 1.3 x T caused a steeper repolarization of the EPSP as a result of disynaptic inhibition from Ib afferents (Eccles et al. 1957) (although recurrent IPSPs could also contribute). The data indicated that both the group Ia and Ib afferents were conducting normally up to 17 days after axotomy of the LGS nerve. This is well after the time that the efficacy of LGS group I afferents was reduced (figure 4-1C).

Discussion

Previous studies have shown that an excitatory pathway is opened from group I extensor afferents during locomotion in cats that causes a facilitation of the ongoing extensor bursts (Pearson and Collins 1993; Guertin et al. 1995) and can also prolong the extensor burst (Guertin et al. 1995) and delay the onset of flexion (Chapter 2). We reported in decerebrate walking cats (Chapter 2) that stimulation of the group I afferents in the LGS nerve could powerfully prolong the extensor burst, while in contrast stimulation of other extensor nerves had relatively modest effects. The main result of this study is that the efficacy of the locomotor-dependent group I excitatory pathway regulating the stance to swing transition in the hindleg of a stepping cat can be altered by cutting the LGS nerve. We observed two main effects after axotomy of the LGS nerve: (1) stimulation of the group I afferents in the MG nerve of the experimental leg was generally more effective in prolonging the extensor burst compared to controls (Figure 4-0) and (4-1) stimulation of the previously cut LGS nerve at group I strengths was generally less effective in prolonging the extensor burst compared to controls (figure 4-1). The reduction in LGS efficacy is probably due to central changes and not due to peripheral factors such as a decrease in the diameter of the group I afferents (figure 4-2).

Previous research on plasticity in reflex systems of the spinal cord has focused on the group Ia monosynaptic pathway because of its relative simplicity. Our results show that plasticity of the locomotor-dependent group I excitatory pathway regulating stance shares some similarities with plasticity of the group Ia monosynaptic pathway. After axotomy of the LGS nerve its group Ia monosynaptic efficacy decreases (Eccles et al. 1953, 1959) and the efficacy of the remaining synergists increase (Eccles et al. 1962) over a period of weeks to months. Gallego et al. (1979) confirmed the results of Eccles et al. (1959) and emphasized that no decreases of homonymous EPSP's occurred in the first week after nerve section. One important difference, however, is that the adaptation of the locomotor-dependent group I excitatory pathway regulating stance appears to be relatively rapid. Increases in MG and decreases in LGS group I efficacy after LGS nerve section can take place within days and are clearly present by the end of the first week. One question for future study would be to investigate if the plasticity in the locomotor-dependent group I excitatory pathway could occur faster than 3 days? Our primary question in this study was to illustrate that plasticity was possible in the excitatory pathway and thus we were not concentrating on establishing a minimum time for the plasticity to occur. To establish an exact time course would be quite difficult given the variability in the amount of plasticity from cat to cat (figure 4-0C & 4-1C).

Another question is whether the plasticity reported in this study is functionally significant? In answering this question it is important to realize that this pathway we have examined may also have a role in regulating the magnitude of extensor activity during stance (Pearson and Collins 1993; Guertin et al. 1995). Also it receives convergent input from group Ia as well as group Ib afferents (Guertin et al. 1995). There is evidence from cats as well as humans that feedback from group Ia afferents contribute to the generation of extensor activity during stance (Severin 1970; Yang et al. 1991). Thus, it would seem to be important to be able to calibrate the gain of any

reflexes involved in regulating motor activity during various locomotor tasks. An attractive possibility, therefore, is that the plasticity we have observed is related to this need to calibrate the gain of reflexes which support the generation of extensor activity. This function would be analogous to the role of plasticity in the vestibular ocular reflex (VOR) for calibrating gain (Lisberger 1988). Interestingly, the gain change in the VOR is also rapid and can recalibrate within a few days (Miles et al. 1985).

In light of the relatively rapid onset of plastic effects in the group I afferents of the MG nerve it is interesting that after chronic axotomy of the LGS nerve most cats exhibited few behavioral changes in their gait pattern. The only visually apparent deficit after axotomy of the LGS nerve was an increase in the yield of the ankle joint during the E_2 phase of locomotion during the first few days (unpublished observations). If feedback from group I afferents does reinforce ongoing extensor activity during stance then it is possible that a rapid recalibration of the MG group I afferent strength could partly mediate this swift recovery of function. Supporting this idea is a result from Gordon et al. (1978) who reported that the amplitude of the MG EMG increases over time after ligation of the LGS nerve for several months. In addition, Wetzel et al. (1973) reported that cats which had their soleus and PL nerves cut quickly recovered full locomotor ability with no deficits in gait (c.f. Webb and Cope 1992). In the future it will be important to establish whether plasticity in spinal reflex pathways has a role to play in functional recovery after peripheral or central lesions of the nervous system.

This study reports that plasticity is possible in the excitatory group I pathway that has access to the pattern generator for locomotion. In the future many questions need to be answered regarding the mechanisms and locus of the plasticity. The most obvious is whether the site of plasticity

resides within the spinal cord. It will be of some interest therefore to repeat this study using acute and chronic spinal locomoting cats.

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Chapter 5

Plasticity of the group I extensor pathway controlling the stance to swing transition: its site and timecourse[5]

Introduction

The central nervous system shows a remarkable ability to adapt to different environmental conditions (Thompson, 1988). Although adaptive plasticity is often thought to occur mainly at higher levels of the neuraxis, plasticity of reflex pathways located within the spinal cord can also occur in response to injury, muscular inactivity, operent and classical conditioning and conduction block of afferent activity (Wolpaw and Carp, 1993, Mendel, 1984). Altered supraspinal or afferent inputs can often contribute to the adaptation of spinal reflex pathways. For example, in the monkey the amplitude of the H-reflex can be operently conditioned to either increase or decrease. Since the reflex remains partly conditioned after spinalization, its locus must lie within the spinal cord. However, differences in the effects have led to the conclusion that the changes in the reflex in the intact monkey were produced by a combination of spinal plasticity and tonic supraspinal influences (Wolpaw and Carp, 1993). In the cat, Spencer and April (1970) have shown that tenotomy of a muscle causes the magnitude of the homonymous H-reflex to increase. When the spinal cord was transected at the same time as the tenotomy this adaptation did not occur suggesting that disuse alone was not responsible for the deficit.

After sustaining neural injuries many animals regain lost functions quite well and the analysis of these behavioral recoveries has provided some clues regarding the function of supraspinal inputs

[5] This chapter has not been published. We intend to submit this paper for publication in the Fall of 1996. As primary author of this chapter, I was heavily involved in the planning, performance and execution of the experiments and I performed all of the subsequent data analysis. Parts of this chapter were edited by Dr KG Pearson.

in mediating the improvement. Goldberger (1977) found that 7-14 days after unilateral deafferentation of a cat it can locomote surprisingly well by relying on cues regarding changes in its centre of gravity caused by misplaced foot falls and information from afferents in the trunk. After the animals had recovered, a second operation was performed to hemisect the spinal cord ipsilateral to the deafferentation. Interestingly, after this procedure the deficits returned and the animals never showed any signs of recovery. Thus, ipsilateral descending tracts were essential for the recovery of locomotion after unilateral deafferentation. It has recently been shown that cats which have their TA and EDL muscles denervated can successfully adapt their gait after a number of weeks. However, if these cats are then spinalized and trained to walk they no longer exhibit signs of the adaptation (Dr. S. Rossignol, Personal Communication).

While there is little doubt that supraspinal inputs can alter reflex pathways, changes also occur in the transected spinal cord (Mendel, 1984; Patterson et al., 1973; Spencer et al., 1966; Kandel, 1981). A vivid behavioral example occurs in chronic spinal cats which can regain the ability to walk with full weight support if they are trained on a daily basis (Barbeau and Rossignol, 1987). It was found that the sooner the training following the transection the better the walking pattern produced (Hodgson et al., 1994), indicating that the circuitry within the spinal cord is especially susceptible to change in the first weeks following transection. While these behavioral studies in the intact and spinalized animal have been extremely useful, they have shed little light on which reflex pathways can be altered after injury to the nervous system. This is because until quite recently the afferent signals that are involved in controlling the step cycle were not known.

Progress has been made in identifying afferent signals that contribute to the control of locomotion (for review see Pearson, 1995; Whelan, 1996). Sensory input from receptors that signal changes in the length of the flexor muscles can lead to the initiation of swing in walking

cats (Hiebert et al., 1996). Similarly, input from receptors that signal changes in length (group Ia) and contractile force (group Ib) in extensor muscles act to maintain the amplitude and duration of stance, ensuring that swing is not initiated while the leg is bearing the weight of the animal. This pathway is only open during locomotion and it consists of an oligosynaptic pathway from extensor group I afferents to extensor motoneurons. It has recently been shown that plasticity of this reflex pathway can occur in walking decerebrate cats (see chapter 4). If the lateral gastrocnemius and soleus (LGS) nerve was cut for 3-28 days before the animal was decerebrated, large changes in the effects of electrically stimulating the LGS or MG nerve at group I strengths occurred. As expected from previous studies, stimulation of the LGS nerve in the control leg, strongly prolonged the extensor burst, while stimulation of the MG nerve had only a weak effect (Chapter 2; Appendix 2). The results from stimulating these nerves in the experimental leg were very different. Stimulation of the previously cut LGS nerve had only a weak effect on the duration of the extensor burst, while in contrast, stimulation of the MG nerve now strongly prolonged stance. One important issue that was not addressed in our previous investigation was whether cats who show signs of plasticity can conserve these adaptations after spinalisation. If this were the case it would indicate that the locus of plasticity is contained within the spinal cord.

In this investigation we have expanded the results presented in our previous publication in three ways. Firstly, we have increased the number of decerebrate animals tested to establish a time course for the increase or decrease in effectiveness of the MG or LGS nerves respectively following axotomy of the LGS nerve. Secondly, we tested whether the locus of plasticity was located in the spinal cord. To test this idea we first of all determined whether plastic changes had occurred in the decerebrate walking animal. If plastic changes had occurred then we transected the spinal cord of the decerebrate cat and induced stepping by adding L-DOPA. We then repeated the protocol of stimulating the extensor nerves in each leg to see if the differences were

conserved in the spinal state. Thirdly, we documented the functional deficits encountered by some of the animals following axotomy of the LGS nerve.

Methods

All animals used in this study were cared for in accordance with the guidelines published by the American Physiological Society and the experimental procedures were approved by the University of Alberta animal welfare committee. Experiments were carried out in 24 adult cats of both sexes.

Chronic Procedures

All 24 animals underwent the following minor surgical procedure: Under Halothane anesthesia and aseptic conditions the nerve supplying the LGS was exposed and transected close to the muscle. The proximal nerve was tied with 6.0 silk to mark it for future identification. An antibiotic (Ayercillin, 1 cc) and, if necessary, an analgesic (Buprenorphine; .005 -.01 mg/kg) were administered for up to 1 week after surgery. Animals were allowed out of their cages on a daily basis after surgery and the cages were large enough (dimensions: 73 cm wide by 69 cm deep by 84 cm high) to permit the animal to move and to jump onto a ledge. After a period of 3 to 34 days, the acute surgical and experimental procedures were performed on the cats.

In 3 of the 24 intact animals, EMG electrodes were implanted to monitor any changes in EMG patterns that occurred after the cut of the LGS nerve. These animals were trained to walk bipedally or quadrupedally for 3 weeks before this procedure, using food rewards as an incentive. The surgical procedure was carried out under Halothane anesthesia and aseptic conditions. The following muscles were implanted in both legs using Cooner wire electrodes (AS632; Cooner Wire Company): vastus lateralis (VL), medial gastrocnemius (MG); semi-tendinosis (St) in 1 animal, tibialis anterior (TA) in 2 animals. All wires were led subcutaneously to a headpiece

manufactured from dental acrylic and held in place by screws implanted into the skull of the animal. The animals were allowed to recover for 3-4 days following this procedure and an analgesic was administered if necessary. After the animals had fully recovered, control EMG responses and kinematics were recorded while the animal walked quadrupedally and bipedally at .4 m/s. We then tracked the functional recovery starting 6 hours after the cutting of the LGS nerve. Video taping of the animals' step cycle and the EMG recordings were made as the animal walked bipedally and quadrupedally. Subsequent recordings were made on a daily basis until the acute surgical procedure began.

Acute Procedures

Only an abridged description of the acute procedure is included here, for a full description please see (Chapter 2 and 4)

Preparation	Decerebrate Only				Decerebrate / Spinal			Spinal Only		
Nerves stimulated	LGS	PL	MG	Total Cats	LGS	MG	Total Cats	LGS	MG	Total Cats
Number	12	3	10	13	9	9	9	2	2	2

Table 5-0: The number of animals used, type of preparation and the nerves stimulated in each animal..

In this study we used 2 types of walking cat preparations to test the effectiveness of extensor group I stimulation; the decerebrate walking cat and the spinalized L-DOPA treated animal. Table 5-0 summarises the number of animals used to test the effectiveness of extensor nerve stimulation for each nerve and the type of preparation used in each case. Halothane anaesthesia was administered to each animal during acute surgery. In all animals the extensor nerves were tied into stimulating cuffs. The stimulating cuffs were manufactured from latex and were each about 6 mm long with an inside diameter of about 3 mm (chapter 2). A silicone recording cuff was placed around each of the sciatic nerves to record the strength of the stimulus. The threshold of the electrical stimulus to the extensor nerves was taken as the minimum voltage necessary to

produce a visually detectable sciatic potential. The strength of the stimulus was expressed in multiples of this threshold level. Bipolar stainless steel recording electrodes (Cooner Wire Company, AS632) were sewn into the following muscles of both hindlegs to record electromyographic (EMG) activity during walking: MG (in the 3 cats which did not have the MG nerve cut), vastus lateralis (VL), semitendinosus (St) and iliopsaos (IP). The wires from both the stimulating and the EMG electrodes were led subcutaneously to a multipolar connector on the back of the cat. After finishing this procedure, the cats were placed over a motorised treadmill. The cats were supported under the abdomen by a sling to maintain lateral stability and to aid in weight support. The animal was then decerebrated by transecting the brainstem at a 50^{o} angle from the anterior edge of the of the superior colliculus using a stereotaxically guided spatula. The Halothane anaesthesia was discontinued at this time. In 21 out of the 22 decerebrate cats spontaneous bouts of walking occurred in response to a moving treadmill. Occasionally, manual stimulation of the perineum was used to evoke these bouts of locomotion. Although the triceps surae were denervated, most cats produced a stepping pattern that was equivalent to that produced by normal decerebrate cats except for a sag of the ankle joint during the E_2 phase of stance. The speed of the treadmill was set between 0.25 - 0.35 m/s, depending on the animals step pattern. During stance, a stimulus train to the appropriate extensor nerve (1.8 - 2 x T) was triggered 200 ms after the onset of the extensor EMG (mostly VL; in 3 initial experiments MG). The duration of the stimulus train was 1000 ms, which was short enough to allow stimulation of a normal LGS nerve to prolong the extensor burst for the duration of the train. Stimulation of a normal MG nerve did not usually prolong stance for a stimulus train of this length. This was important because it allowed for reductions in LGS efficacy *and* increases in MG efficacy to be observed in the experimental limb.

After we had completed the decerebrate protocol (1-3 hours), we spinalized 9 of the decerebrate cats at the T12 level. We then infused nialamide (50 mg/kg) dissolved in 20 ml of water into the animal over a period of 45 minutes. The nialamide (Sigma chemicals) was dissolved in an acidic medium (HCl) and then buffered (NaOH) to a pH of approximately 6.3-7.0. After the nialamide had been administered, we infused methyl ester L-DOPA (50 mg/kg) (Sigma chemicals) dissolved in 5 ml of water. In approximately 20-30 minutes after the infusion of L-DOPA, various degrees of rhythmic behavior were observed in the spinal animals. During periods of rhythmic behavior, the LGS and MG nerves, from each hindlimb were stimulated using similar stimulus parameters as in the decerebrate state.

Data analysis

All data were recorded using a Vetter 4000A PCM recorder. Later, selected sequences were stored on computer disc using the Axotape (Axon Instruments) data acquisition system installed on a Microexpress 486 computer. Data analyses were carried out using custom programs that could retrieve data from the axotape files. The cycle periods before, during, and after the stimulus were calculated only during regular sequences of walking. Each cycle period was calculated as the time between the occurrence of successive St or Ip bursts. A spreadsheet program (Microsoft Excel 5.0) was used to calculate the mean and standard deviation for these cycle periods and Student's t-tests were administered to detect significant differences between the conditions. The data were normalised according to the equation below to allow for comparisons between cats and between control and experimental nerves.

Equation 5-0:

Percentage effectiveness = [(b-a)/(c-a)] x 100.

Where *b* equals the stimulated cycle period, *a* represents the control cycle period and *c* represents

the time from the first flexor burst before the stimulated extensor burst to the offset of the stimulus train (see Fig 5-0A for an illustration of what the variables measured). The percentage effectiveness is a measure of how powerfully the stimulus could affect the step cycle. For example, if the stimulus was 100% effective, the next flexor burst would be held off until the end of the stimulus train. By contrast, if the percentage effectiveness was 0% the stimulus would have no effect on the cycle period.

All kinematic data, including stick figures, were obtained by analysing selected video sequences using Video Blaster software (Creative Labs) and custom-designed software. The measurement of mean yield around the ankle joint was calculated by measuring the maximum extension of the ankle at the end of the E_1 phase just as the foot touched the ground and subtracting the minimum angle of the ankle joint during the E_2 phase for 4 consecutive steps. In 3 animals, we measured any changes in the extensor EMG bursts that occurred following axotomy of the LGS nerve. The increase in the EMG was calculated by integrating a set area under the EMG curve (200 ms in length). Usually 20 steps were used to calculate the mean and standard deviation of the integrated area under the curve.

Results

In chapter 4 we reported that axotomy of the LGS nerve in an otherwise intact cat could decrease the ability of the group I afferents in this nerve to prolong stance. Stimulation of the MG nerve at group I strengths, which normally has a modest effect on the timing of the step cycle, had a larger than expected effect on the timing of the step cycle following axotomy of the LGS nerve. A positive test for plasticity was judged to occur if group I stimulation of the LGS or MG nerves had significantly different effects on prolonging the step cycle when compared to stimulation of the same nerves in the control leg.

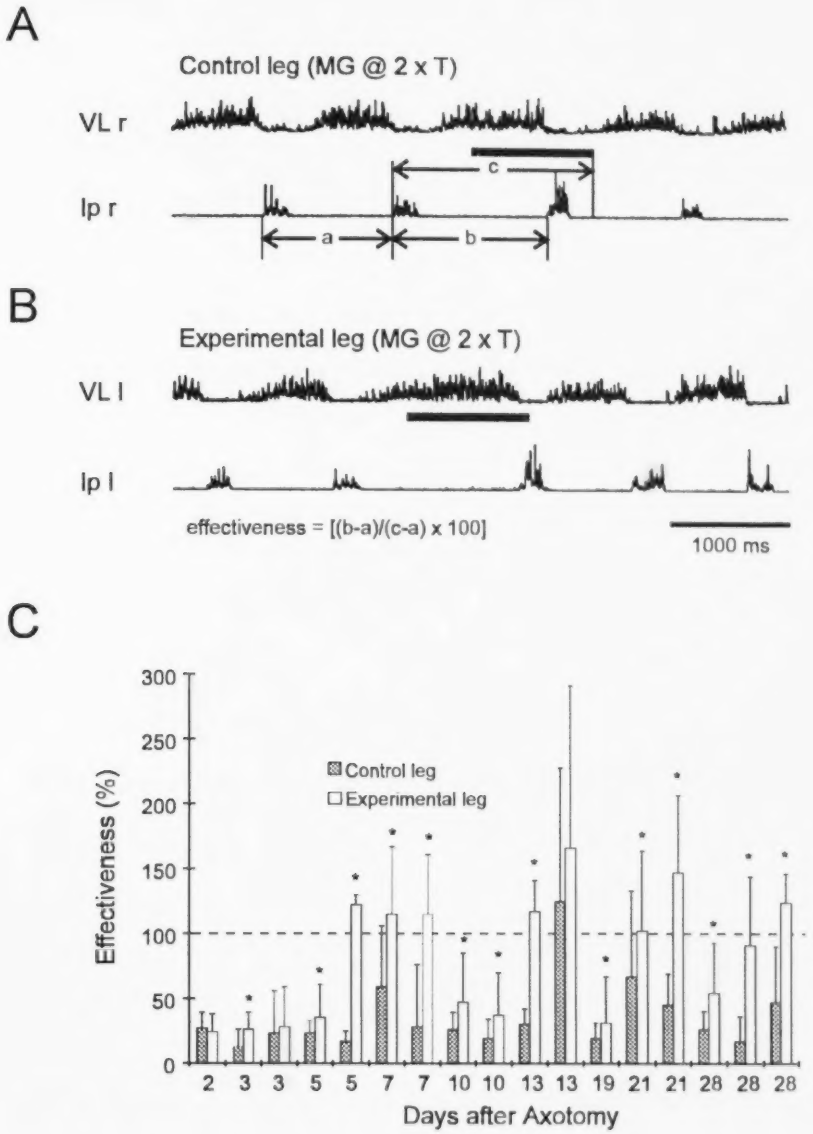

Figure 5-0: Axotomy of the lateral gastrocnemius and soleus (LGS) nerve increases the ability of group I afferents in the medial gastrocnemius (MG) to prolong extensor burst activity A and B: rectified and filtered electromyographs (EMGs) from the hind leg muscles showing individual trials in the control (A) and experimental (B) legs (stimulus trains: 1000 ms duration, 200 Hz, 2 x T). Note that in the control leg (A), stimulation of the MG nerve modestly increases the duration of the extensor burst. In contrast after axotomy of the LGS nerve for 21 days, stimulation of the MG nerve using similar parameters of stimulation results in a relatively large increase in the duration of the extensor burst. C. bar graph showing the mean percent effectiveness of MG stimulation of the control and experimental legs for each individual experiment. Error bars represent the standard deviation. Asterisk indicates that there is a significant difference between the two conditions ($P < .05$). The number on the abcissa below each bar indicates the duration of the axotomy. *VL l, vastus lateralis, left leg; Ip l,iliopsoas left leg; VL r, vastus lateralis, right leg; Ip r, iliopsoas, right leg*

Comparison of the responses elicited in each leg upon stimulation of the MG, LGS or Pl nerves

We reported that the effectiveness of stimulating the MG nerve increased if the LGS nerve was cut for 5-28 days before the acute procedure (chapter 4). Similarly a decrease in the effectiveness of the previously axotomized LGS occurred in as few as 3 days when compared to the control leg. One issue in that paper was whether the effectiveness of stimulating the LGS or MG progressively changed following axotomy of the LGS nerve. In our previous study no clear trends were present in our data. To improve the likelihood of finding a time course for the decrease in LGS and increase in MG effectiveness we have pooled the data from our previous investigation with the data obtained in this study.

Figure 5-0C shows the difference in effectiveness of stimulating (trains: 1000 ms duration; 2 x T; 200 Hz) the experimental and control MG nerves in all the animals tested. Despite the variability in effects from animal to animal, it is clear that stimulation of the MG nerve in the control leg (gray bar) had a modest effect on the duration of the step cycle (figure 5-0B, 5-0C). Stimulation of the MG nerve in the experimental leg (white bar) prolonged the step cycle for a significantly greater period of time in 14 out of 17 cats (figure 5-0A, 5-0C). These changes could occur as soon as 3 days following axotomy of the LGS nerve, however, the strongest effects were observed after the nerve had been axotomized for 5 days or longer. To establish whether an increase in the effectiveness of stimulating the experimental MG nerve was dependent on the time of the axotomy of the LGS nerve, we took the data presented in 1C and plotted a standard X-Y plot with time of axotomy as the abscissa and the effectiveness of the stimulus train as the ordinate. The data obtained from stimulating the MG nerve in the control leg shows that, as expected, there was no tendency for stimulation of the control MG nerve to increase following axotomy of the contralateral LGS nerve (figure 5-1B). In contrast, stimulation of the MG nerve

in the experimental limb containing the previously cut LGS nerve often increased the duration of the extensor burst for the duration of the stimulus train or longer (figure 5-1A). As can be seen from figure 5-1A, there was no trend for a progressive increase in the effectiveness of stimulation of the MG nerve following axotomy. However, significant increases in the effects of stimulating the MG nerve were produced 5 days after axotomizing the ipsilateral LGS nerve.

Figure 5-2C shows the effectiveness of stimulating the LGS nerve (trains 1000 ms; 2 x T; 200 Hz) in the control and experimental legs for all the animals tested. A significant reduction in the effectiveness of stimulating the LGS nerve in the experimental leg occurred 3 days after the nerve was cut. In 1 animal tested at 2 days, no significant change in effectiveness ($p > .1$) was found. Overall the effectiveness of stimulating the LGS nerve in the experimental leg was significantly reduced in 15 out of 18 cats. When the data presented in figure 5-2C were plotted (in a similar fashion as the MG data), a noticeable decrease in the effectiveness of stimulating the LGS nerve occurred as the time following axotomy increased (figure 5-1C). As expected, stimulation of the LGS nerve in the control leg (figure 5-1D) tended to prolong the step cycle for the duration of the stimulus train (100% effectiveness).

In 3 cats, we tested whether stimulation of the plantaris nerve (the MG nerve was left intact) had an increased effect on the duration of the MG or VL extensor burst following axotomy of the LGS nerve (tested at day 3, 7 and 14 respectively). Normally, stimulation of the Pl nerve has a modest effect on the duration of the step cycle that is very similar to equivalent stimulation of the MG nerve (Chapter 2; Appendix 2). In none of the animals did we detect any increase in the effectiveness of stimulating the Pl group I afferents in the experimental compared to the control leg.

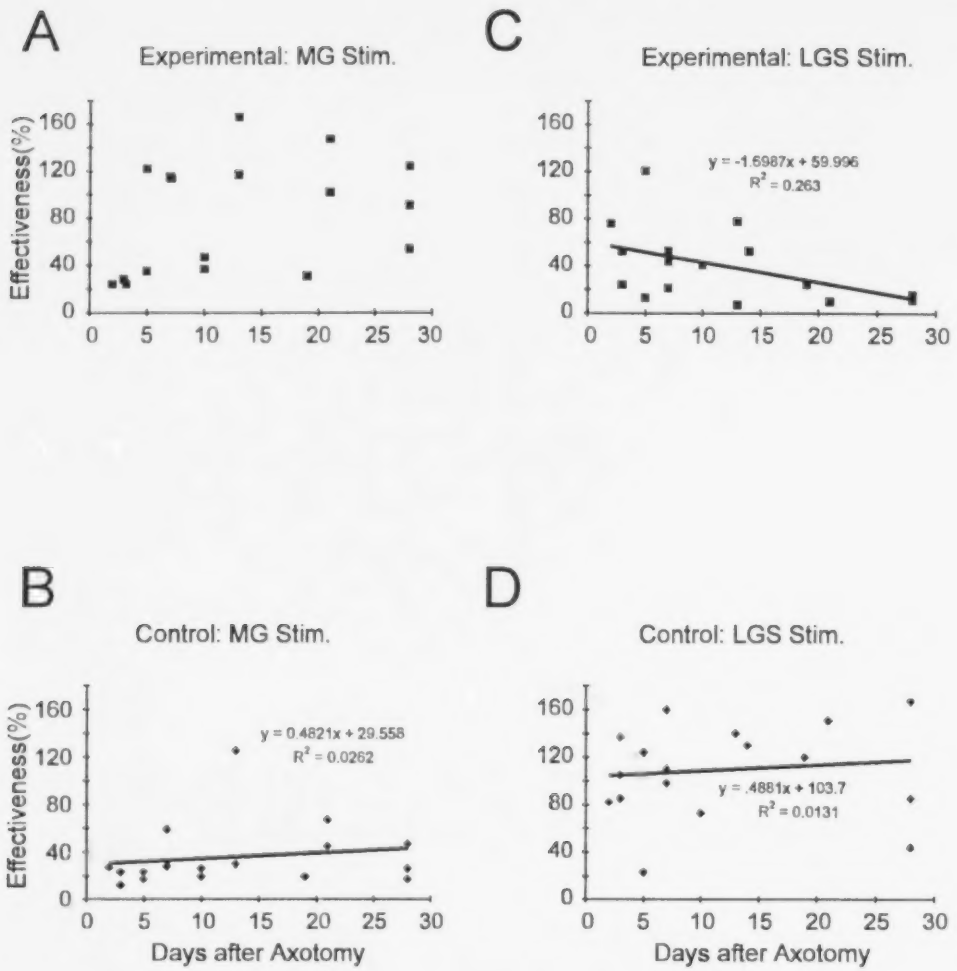

Figure 5-1: Stimulation of previously axotomized extensor nerves or their synergists have altered effects compared to stimulation of similar nerves in the control leg. A,B,C,D Scatterplots of the data in figures 5-0C and 5-2C with the abscissa indicating the number of days following axotomy and the ordinate indicating the effectiveness of the stimulus. Best fitted linear regression lines are shown along with the regression coefficient for each graph except for (A) in which the data were too scattered to do so. Each data point indicates the mean effectiveness from an individual experiment. A, Data from stimulating the experimental MG nerve for all experiments showing that a rise in effectiveness took place on the 5th day which resulted in the stimulus train prolonging stance frequently for the duration of the train (100% effectiveness). B. Stimulation of the MG in the control leg frequently had a modest effect on the duration of the stimulus train (y-intercept=29.55%) which did not increase with time. C. Stimulation of the LGS group I afferents in the experimental leg showed a gradual decline in effectiveness which was dependent on the time following axotomy of the LGS nerve. D. Stimulation of the LGS group I afferents in the control leg had a large effect on the duration of stance (y-intercept 103.7%) which did not increase with time.

Axotomy of the LGS nerve reduces effects of group I afferents on the amplitude of the MG bursts

A proposed role of extensor group I afferents is to reinforce the extensor burst during the stance phase (Pearson and Collins, 1993). In previous experiments we have found that stimulation of the group I afferents in the LGS nerve has an extremely powerful effect on increasing the magnitude of the MG burst. Thus, we were interested in whether the magnitude of these effects on the amplitude of the MG extensor burst would decrease following axotomy. In 3 animals, we compared the effects of stimulating the LGS nerve in the control and experimental leg on increasing the amplitude of the MG EMG burst (MG nerves were left intact). Normally, stimulation of the LGS nerve in the control leg had a large effect on the amplitude of the extensor burst (figure 5-3A). In contrast, stimulation of the previously axotomized (figure 5-3B) LGS nerve only weakly increased the amplitude of the extensor burst. Similar decreases in the amplitude of the MG EMG were observed in the animal that had its LGS nerve axotomized for 7 days. No effects on either amplitude or duration were observed in the 1 animal in which the LGS nerve was axotomized for 3 days.

Effects of spinalization on expression of plasticity

The locus for the plasticity observed in the decerebrate cat may have been contained in the spinal cord or elsewhere in the brainstem or cerebellum. To test whether the locus was contained within the spinal cord we used the following protocol. First, we tested in 9 decerebrate animals whether there were differences in the effectiveness of stimulating the LGS or the MG nerve when compared to stimulation of the nerves in the control leg. If there were positive signs of plasticity in the decerebrate walking animal we spinalized that animal and attempted to repeat the protocol of stimulating the LGS or MG nerves in the control and experimental legs during spinal locomotion. The locomotor rhythm was induced in the spinal animal by injecting nialamide

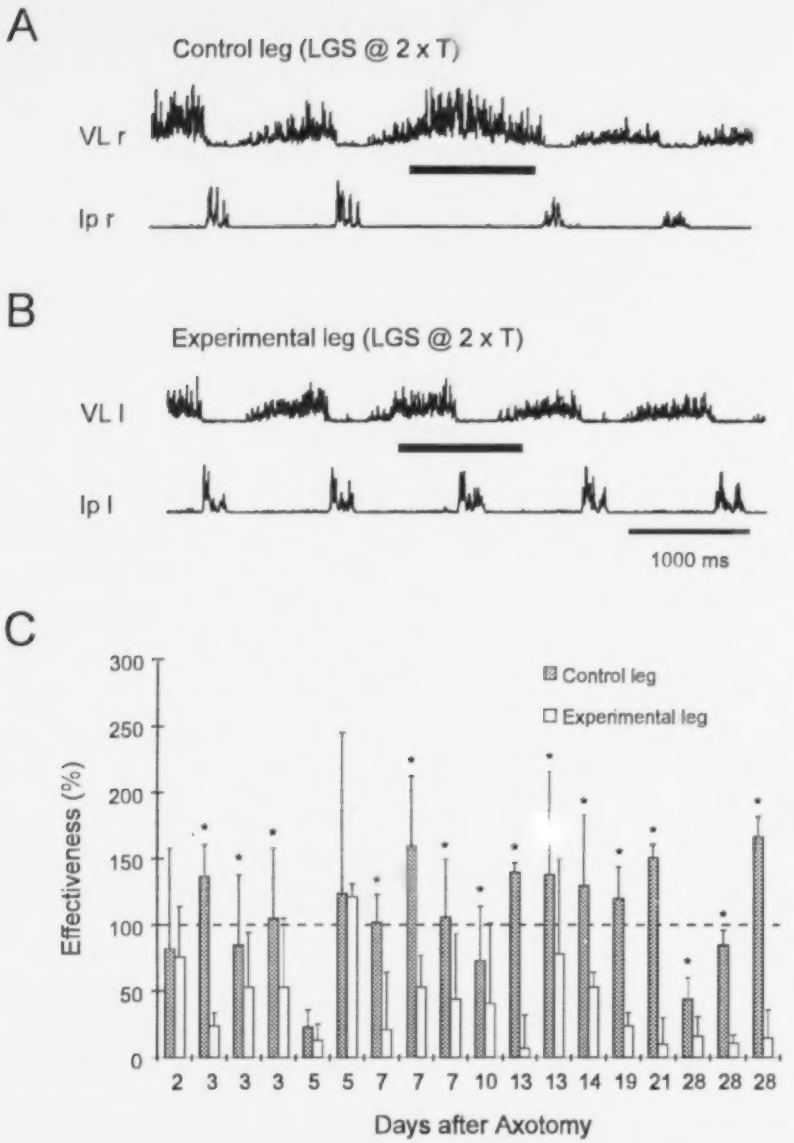

Figure 5-2: Axotomy of the LGS nerve reduces the ability of group I afferents in the LGS nerve to prolong extensor burst activity. A,B Rectified and filtered EMG from hindlimb muscles showing individual trials in the control (A) and experimental (B) legs (stimulus trains: 1000 ms duration, 200 Hz, 1.8 x T). Note that in the control leg (A) stimulation of the LGS nerve produced a large increase in the duration of the extensor activity for the duration of the stimulus train. In contrast in the experimental leg (B) after axotomy of the LGS nerve for 21 days, stimulation of the nerve using similar parameters only modestly increased the extensor burst. C. Bar graph showing the mean percent effectiveness of LGS stimulation of the control (shaded bar) and experimental (open bar) legs for each individual experiment. The error bars represent the standard deviation. The asterisk indicates that there is a significant difference between the two conditions ($P < .05$). The numbers on the abscissa under each bar indicate the duration of the axotomy. *VL r vastus lateralis, right leg; Ip r iliopsoas, right leg; VL l vastus lateralis, left leg; Ip l iliopoas, left leg*

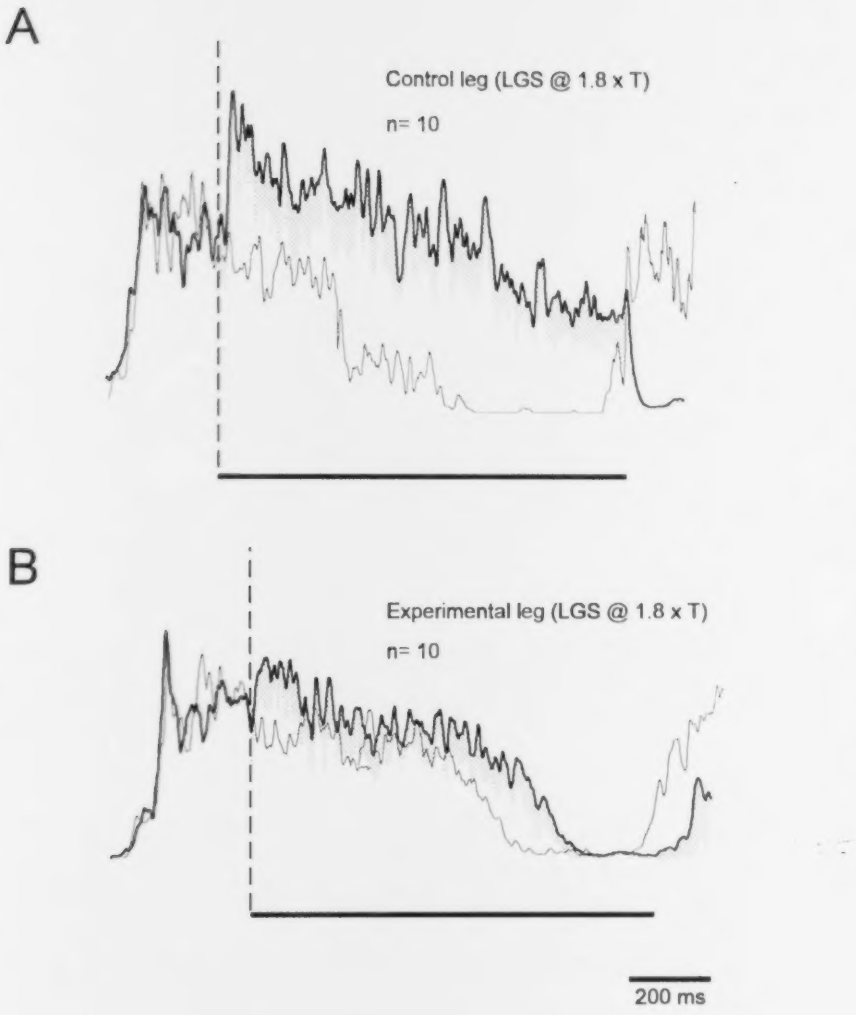

Figure 5-3: Axotomy of the LGS nerve reduces the effects of stimulating the group I afferents in LGS on the amplitude of the MG EMG burst (LGS cut for 14 days). A,B Rectified and filtered EMG traces. The heavy line indicates the stimulated trial and the light line indicates the mean of the EMGs of the unstimulated step taken directly before the stimulated trial. The shaded area beneath the heavy line indicates the 95% confidence interval of the stimulated EMG trial. A, These set of traces show the large effects on the amplitude of the MG EMG produced by stimulating the LGS group I afferents in the control leg. B, By contrast stimulating the LGS group I afferents had a relatively weak effect on the amplitude of the MG EMG in the experimental leg.

followed by L-DOPA systematically. Generally speaking, the rhythm produced in the spinalized animal did not result in stepping movements that were as powerful as those obtained in the decerebrate state. In only 1 animal did we obtain stepping in the spinal state that was comparable to that produced in the decerebrate state. Activation of the flexor muscles were not sufficient to allow clearance of the limb off the ground and bring the limb forward. Even though the stepping was poor in the spinal state, the timing of the extensor and flexor EMG bursts were normal during periods of rhythmicity. In all animals that were spinalized, stimulation of the extensor nerves at group I strengths tended to prolong stance and delay the onset of the next flexor burst. Thus, the qualitative effects of stimulating group I extensor afferents were conserved following spinalization (see figure 5-4A for MG and figure 5-5A for LGS).

Figure 5-4 summarizes the results of stimulating the MG nerve in the control and experimental legs during decerebrate and spinal locomotion for all the animals tested. In 8 out of 9 decerebrate animals we recorded an increase in the effectiveness of stimulating the group I afferents in the MG nerve, however, we were only able to elicit a locomotor rhythm in 5 out of 8 of these animals following spinalization. In 2 out of these 5 animals, the differences between stimulation of the MG nerve in the control and experimental limb persisted following spinalization. However, no significant differences were observed in the remaining 3 animals. In 1 additional animal we carried on with the spinalization even though no differences between stimulating the MG nerve in the experimental or control legs could be found during decerebrate walking (data not shown). As expected, no differences in effectiveness between the MG nerves could be found during spinal locomotion in this animal.

In 9 out of 9 decerebrate animals, we recorded a decrease in the effectiveness of LGS stimulation and were able to repeat the decerebrate protocol in 4 of these 9 animals during bouts of

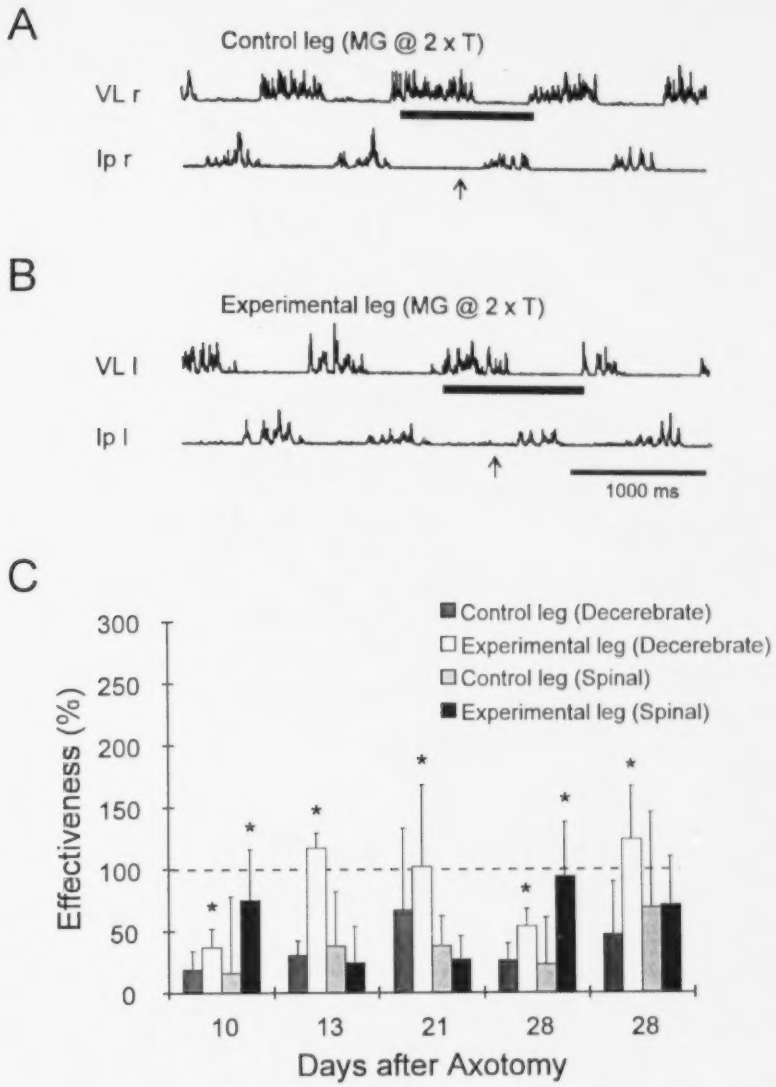

Figure 5-4: Axotomy of the LGS afferents increases the effectiveness of stimulating the MG group I afferents in the decerebrate walking cat but this effect was not always conserved following spinalization. A,B. Rectified and filtered EMG traces (knee extensor VL and hip flexor Ip) from a stepping L-DOPA spinal cat. A, Note that in the control leg stimulation of the MG group I afferents prolonged the extensor burst and delayed flexion for a modest period of time. B, The effects of stimulating the experimental MG nerve in the same animal during L-DOPA induced stepping. Note that the effect on the duration was similar to that shown in A (arrows in A and B point to the expected onset of the flexor burst in the absence of stimulation). C, Bar graphs summarizing the effects of stimulating the MG group I afferents in the experimental and control leg during decerebrate and spinal walking for each animal tested. Note that only 2 out of 5 animals showed a conservation of the changes in effectiveness following spinalization. The asterisks indicate that there was a significant difference between the two legs ($P < .05$). The error bars indicate the standard deviation. The numbers on the abscissa indicate the duration of the axotomy of the LGS nerve. *VL l vastus lateralis left; Ip l iliopoas left;VL r vastus lateralis right; Ip r Iliopsoas right.*

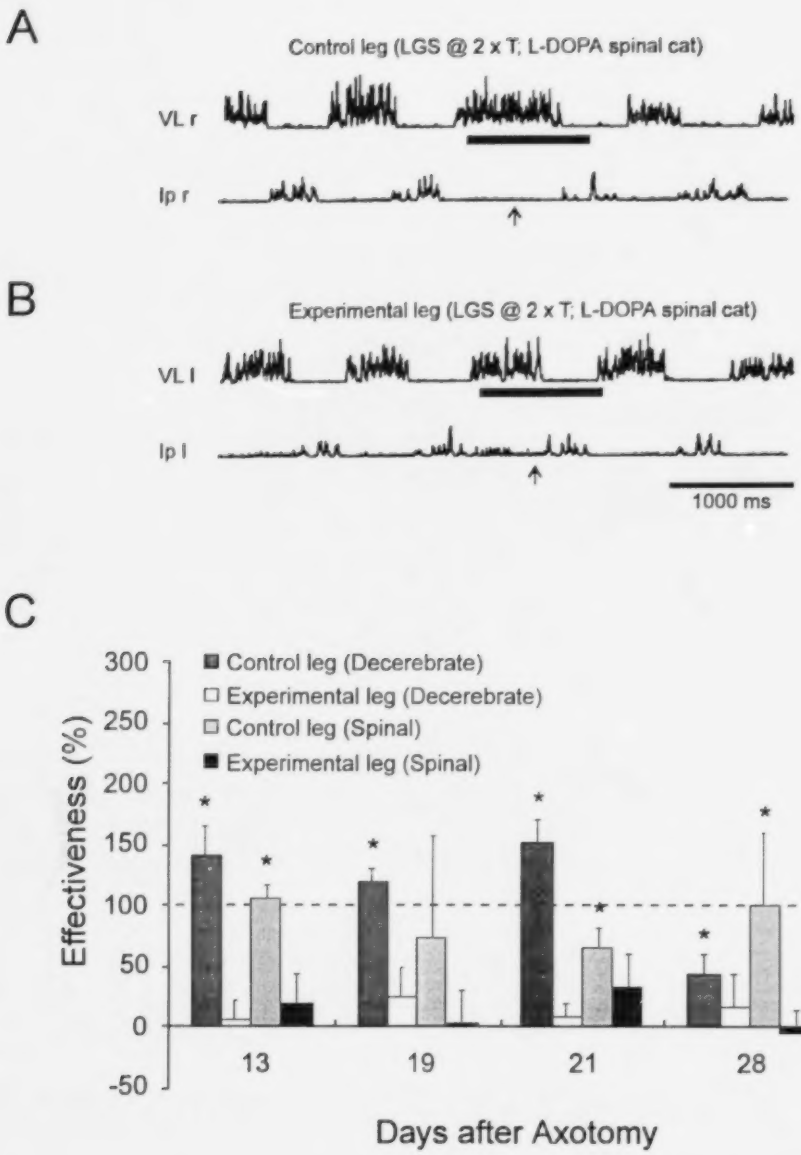

Figure 5-5: Axotomy of the LGS afferents decrease the effectiveness of stimulating the LGS group I afferents in the decerebrate walking cat and this effect was conserved following spinalization. A,B. Rectified and filtered EMG traces from a stepping L-DOPA spinal cat. A, Note that in the control leg stimulation of the LGS group I afferents delayed flexion for a relatively long period of time. B, The effects of stimulating the experimental LGS nerve in the same animal during L-DOPA induced stepping. Note that the effect on the duration was modest compared to that shown in A (arrows in A and B indicate the expected onset of the flexor burst in the absence of stimulation). C, Bar graphs summarizing the effects of stimulating the LGS group I afferents in the experimental and control legs during decerebrate and spinal walking for each animal tested. Note that 4 out of 5 animals showed a conservation of the changes in effectiveness following spinalization. The asterisks indicate that there was a significant difference between the two legs ($P < .05$). The error bars indicate the standard deviation. The numbers on the abscissa indicate the duration of the axotomy of the LGS nerve.

rhythmicity in the spinal state. In all 4 of the animals tested the difference in effectiveness between the control and experimental leg persisted following spinalization. Figure 5-5A shows that stimulation of the control LGS nerve at group I strengths prolonged stance for the duration for a long period and clearly delayed the onset of the flexor burst.

Analysis of the deficits in gait and changes in EMG activity that occurred following axotomy

We had observed in our previous report (chapter 4) that most animals recovered remarkably quickly following axotomy and all could walk 4-5 hours following surgery. Following axotomy of the LGS nerve there was an increased yield around the ankle joint as the animal stepped. This yield was rarely visually apparent after one week suggesting that the remaining extensors of the ankle had increased their activity to compensate for the injury. To quantify the yield of the ankle and the subsequent reduction in this yield we trained 3 animals to walk bipedally and quadrupedally on a treadmill. After this training was complete we implanted EMG recording electrodes into various extensor and flexor muscles and allowed the animal to recover for 1 week. After recovery we recorded control EMG and kinematic data from these animals during bipedal and quadrupedal walking at .4 m/s. We then cut the LGS nerve and compared the resultant deficits with the control data. In an additional animal we recorded only kinematic data.

In all of these 4 animals we began recording data as soon as 6 hours following axotomy of the LGS nerve and continued monitoring the cats on a regular basis until we performed the acute procedure to test for plasticity. In all 4 animals we recorded an increased yield of the ankle (and also the knee) joint following axotomy of the LGS nerve. The yield of the ankle differed depending on the locomotor task (comparison completed in three of the four animals). When the animals were walking bipedally, the deficit was mild (generally causing an increased flexion around the ankle of between 5-10 degrees compared to normal). In contrast, the yield was much

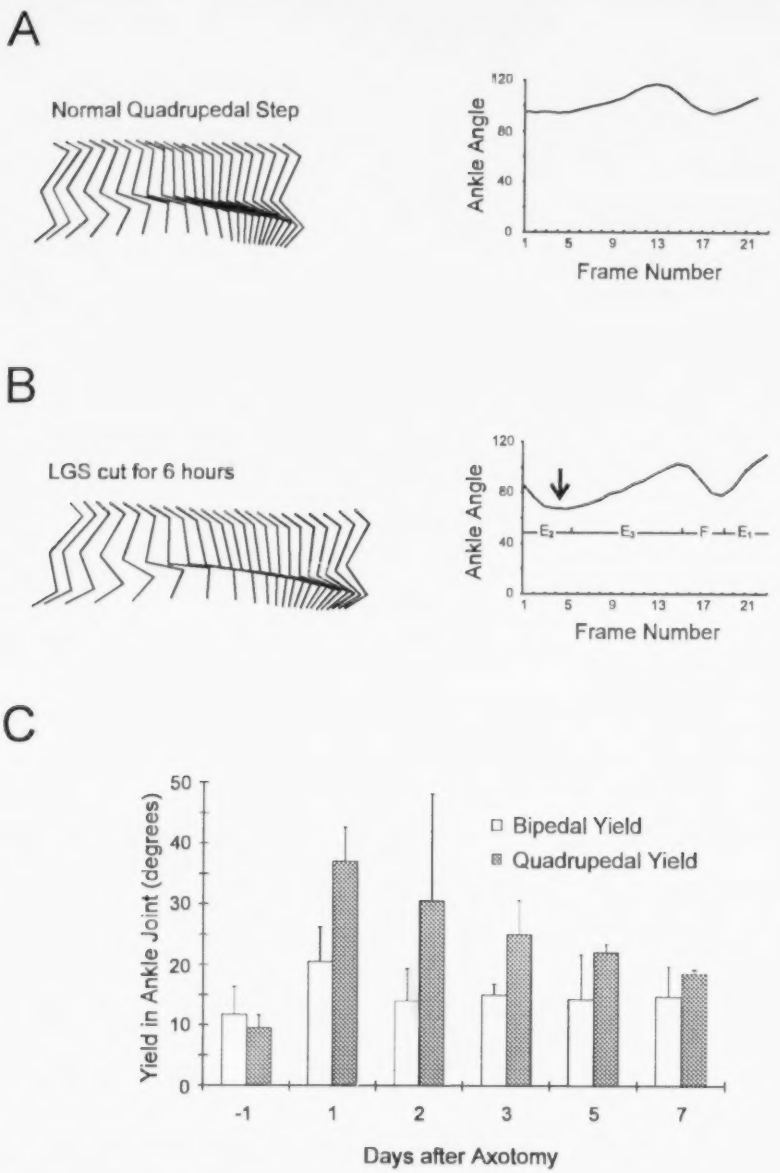

Figure 5-6: Recovery of a single animal following axotomy of the LGS nerve. A,B Stick figures and accompanying ankle angle plots showing a single representative step starting from contact of the foot with the ground. A, The stepping behavior of the animal walking quadrupedally walking at .4 m/s on a treadmill before axotomy of the LGS nerve. B, The same animal walking at the same speed 6 hours following axotomy of the left LGS nerve. Note the increased flexion of the ankle during the stance phase and especially at the beginning of stance. The ankle angle plot clearly shows the increased yield of the ankle that occurred after axotomy (each video frame is approximately 30ms in length). C. Bar graphs showing the mean yield of the ankle joint during the E_2 phase of the step cycle as the animal was walking bipedally or quadrupedally. Note that the yield of the ankle was noticeably increased on day 1 and that it was much greater during quadrupedal stepping. Note that by day 7 the yield during quadrupedal walking was substantially reduced. The error bars indicate the standard deviation.

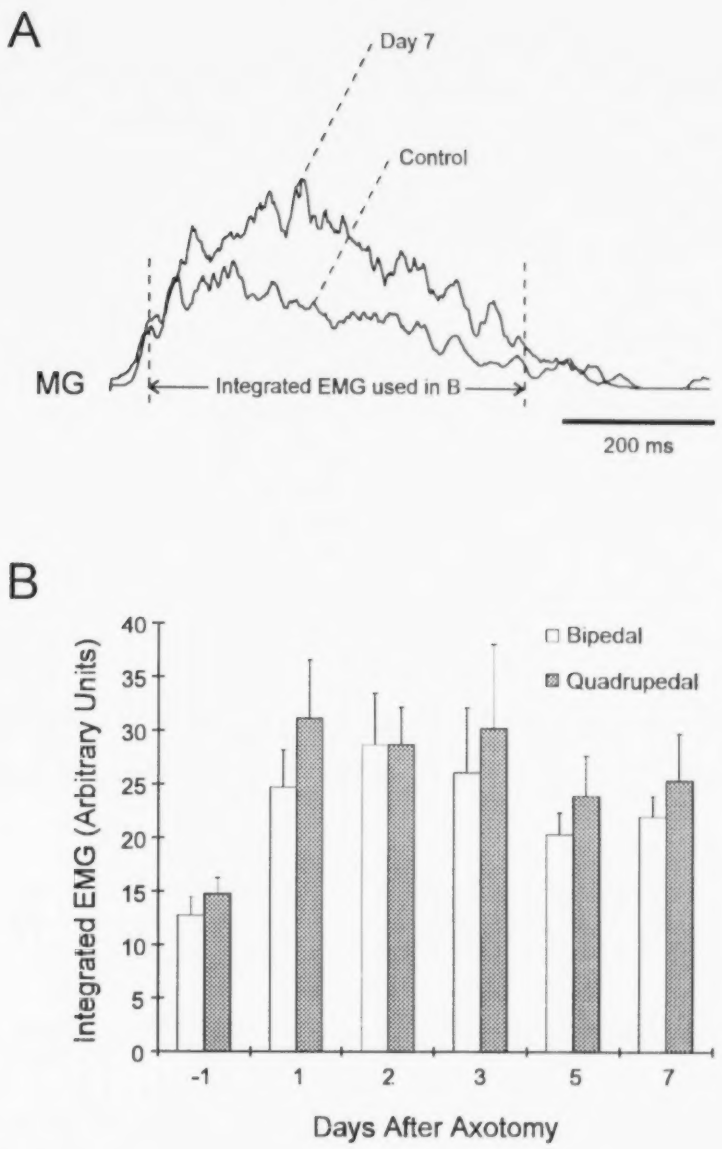

Figure 5-7: The amplitude of the MG EMG increases following axotomy of the LGS nerve. A, Rectified and filtered EMG traces from one animal before (n=5) and 7 days after (n=6) axotomy of the LGS nerve as the animal was walking quadrupedally on a treadmill at .4 m/s. B, The bar graphs show the mean integrated EMG for MG during both quadrupedal and bipedal walking for a single animal. The integrated EMG chosen is shown in A. Note that the amplitude increased significantly as soon as the nerve was axotomized and the increase was similar for both quadrupedal and bipedal walking.

more apparent during quadrupedal stepping, and most animals did not fully recover from this deficit before the acute procedure. Figure 5-6B shows an example of the increased yield of the ankle present 6 hours following axotomy of the LGS nerve while the animal was walking quadrupedally. Note the progressive reduction in the magnitude of the yield that occurred during the first week following the cut of the LGS nerve (figure 5-6C). While the yield of the ankle did reduce over time in two of the three animals monitored clear deficits remained after 3 and 5 days respectively. In the third animal there was no reduction in the yield of the ankle over three days.

In all 3 animals an increase in the amplitude of the MG EMG occurred during undisturbed locomotion following axotomy of the LGS nerve (figure 5-8C,D). This increase occurred during both bipedal and quadrupedal stepping. Figure 5-7 shows data from one animal illustrating the increase in the integrated EMG that occurred following axotomy of the LGS nerve (quantified in figure 5-7B). During bipedal walking there was a progressive increase in the amplitude of the MG EMG in all animals during the first two days after axotomy. After reaching this peak it declined in two animals and declined and then rose again in the other. In all three animals an increase in the amplitude of the VL EMG occurred that was especially pronounced during the first day following axotomy of the LGS nerve. In one animal this increase was sustained for 3 days, while in the other two animals the amplitude declined within 3-4 days following axotomy.

Cat #	Days following Axotomy	MG effectiveness (%)	Increase in MG or VL EMG?	Yield of ankle at time of sacrifice - precut yield (Quad)	Yield of ankle at time of sacrifice - precut yield (Bipedal)
1	3	24	No EMGs	27 (27) degrees	Not studied
2	3	26	MG and VL	14 (19) degrees	8 (8)
3	5	35	MG	19 (23) degrees	14(21)

4	7	115	MG	9 (21) degrees	3 (9)

Table 5-1: Effects of cutting the LGS nerve in intact cats. This table compares the recovery of function of the intact cat with the plasticity of the group I MG pathway observed after the animal was tested in the decerebrate state. The calculation to assess the recovery of function involved taking the yield of the ankle during the last day in which we recorded data from the animal and subtracting the yield before the axotomy of the LGS was made. The numbers in brackets refer to the maximum deficit that occurred during the time that we observed the animal. By comparing both figures one can get a sense of the functional recovery. For example cat 1 did not recovery function, while animal 4 had the greatest degree of functional recovery. Note that the plasticity of the MG group I pathway was correlated to the recovery of function. Increase in MG or VL EMG refers to whether a significant increase in the integrated EMG existed at time of sacrifice compared to precut values ($P<.05$).

In the four animals we were able to see if changes in the strength of the MG group I pathway occurred in the four animals in which we collected kinematic measurements. As can be seen in table 5-1, the effectiveness of stimulating the MG group I afferents was greatest in the animal that was monitored for 7 days following axotomy of the LGS nerve. This animal also showed the largest reduction in the yield of the ankle. In contrast in the animal that we monitored for only 3 days (cat # 1 in table 5-1) there was no recovery in the yield of the hindlimb and no increase in the effectiveness of stimulating the MG group I afferents. One animal showed no increase in the effectiveness of stimulating the MG group I afferents yet showed a modest reduction in the yield of the hindlimb. The last animal showed a modest reduction in the yield of the hindlimb accompanied by a modest increase in the effectiveness of stimulating the MG group I afferents.

Hypertrophy and atrophy of the extensor ankle muscles following axotomy of the LGS nerve

In all of our animals (including animals that did not produce any data) we weighed the lateral gastrocnemius, soleus, medial gastrocnemius and the plantaris muscles from the control and experimental legs. Figure 5-9 shows a plot of the percentage increase or decrease in weight of the muscles of the experimental leg as a function of time. There was a significant atrophy of the LG muscle in the experimental leg that tended to increase with time following axotomy of the LGS nerve. The decrease in the weight of the LG muscle exceeded 60% in one animal that had its LGS nerve cut for more than 30 days. Atrophy of the Sol muscle also occurred but was not as

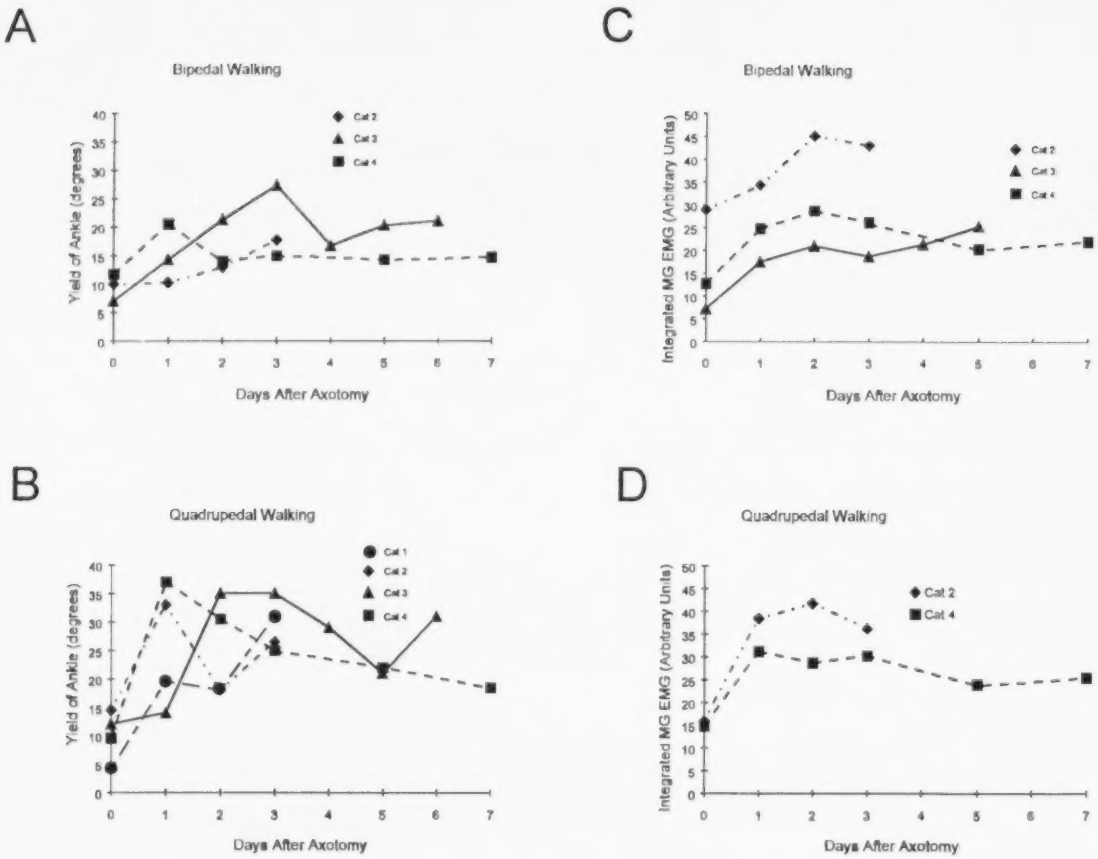

Figure 5-8. An increase in the yield of the ankle was accompanied by an increase in the MG EMG. A,B Line charts showing the mean yield of the ankle joint during the E 2 phase of the step cycle as the animal was walking bipedally (n=4) (A) or quadrupedally (n=2) (B). Note that the deficits were greater during quadrupedal walking for all animals tested. C,D Line charts showing the mean integrated area under the MG EMG during bipedal (n=20) (C) and quadrupedal (n=5-8) (D) walking. Note that the amplitude of the MG EMG was increased after axotomy in all animals. The numbers identifying each cat are the same as those in table 5-1.

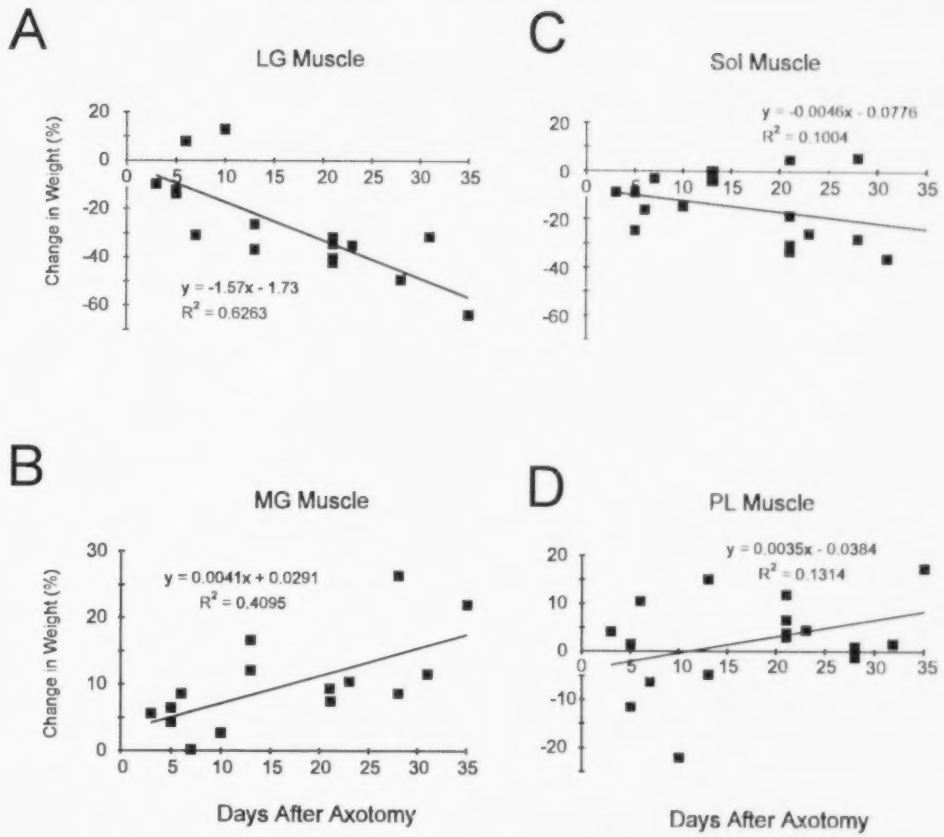

Figure 5-9: Changes in the weights of the MG, Pl, LG, and Sol muscles following axotomy of the LGS nerve. A,B,C,D Scatterplots showing the % change in the wet weight of each muscle following axotomy of the LGS nerve. Each data point represents an individual experiment. The heavy line indicates the best fitting linear regression line. A, The weight of the LG muscle decreased after axotomy of the LGS nerve. B. The weight of the MG muscle in the experimental leg increased following axotomy of the LGS nerve. C, The weight of the Sol muscle decreased slightly. D, The weight of the Pl muscle increased slightly following axotomy. *LG, lateral gastrocnemius; Sol, soleus; Pl, plantaris; MG, medial gastrocnemius.*

pronounced as that observed in LG. Hypertrophy of the MG and PI occurred following axotomy of the LGS nerve. This was most clear for the MG muscle with increases exceeding 20% after 34 days. Even after as few as 5 days following axotomy, the weight of the MG muscle increased by approximately 5-8%.

Discussion

In chapter 4 we demonstrated plasticity could develop in the extensor group I pathway following axotomy of the LGS nerve. Stimulation of the previously cut LGS nerve at group I strengths resulted in the duration of stance being prolonged for a reduced period of time compared to similar stimulation of the freshly cut contralateral LGS nerve. In contrast, stimulation of the MG nerve in the leg containing the previously cut LGS nerve prolonged stance for an increased amount of time than stimulation of the MG nerve in the contralateral leg. In this chapter we have shown that after the LGS nerve is cut, there is a time-dependent reduction in the effects of stimulating the LGS group I afferents on prolonging the duration of the extensor burst. Secondly, we have found that while the decrease in the strength of the LGS group I pathway is conserved after spinalization, the strength of the MG group I pathway became more variable following transection of the spinal cord. Thirdly, following axotomy of the LGS nerve the reduction in the yield of the ankle during stance followed a similar time-course as the appearance of the plasticity in the MG group I pathway.

The parameter we used to measure whether changes in the strength of group I pathways took place was the degree to which stimulation of the extensor group I afferents (MG or LGS nerve) prolonged the step cycle following axotomy of the LGS nerve. Since the observed effects were on the *timing* of the step cycle, we assume that the changes in reflex strength were taking place between group I afferents and the network of interneurons which comprise the extensor half-centre (Pearson et al., 1992; Conway et al., 1987; Gossard et al., 1994). Since the extensor half-

centre is thought to globally excite the extensor motoneurons it would be expected that the amplitude and the duration of the extensor burst would also be prolonged. This was indeed the case for the decline in the strength of the LGS group I pathway. Stimulation of LGS group I afferents that had been previously axotomized had a reduced effect on the amplitude and the duration of the MG extensor burst. However, stimulation of the MG group I afferents only increased the duration of the step cycle and not the amplitude of the VL extensor burst. The likely reason for this was that we were recording the extensor EMG from VL and not the ankle extensors EMG (since they were denervated in these experiments) following stimulation of the MG group I afferents. This is because stimulation of ankle extensor group I afferents has a greater effect on the amplitude of the ankle extensors than on extensors of the hip or knee (Guertin et al., 1995; Chapter 3), therefore any changes on the amplitude of the extensor burst would be expected to be most dramatic in the ankle extensors. Although the effects of stimulating the MG group I afferents had an increased effect on the timing of the extensor burst, the amplitude of the ankle extensor burst may not necessarily be increased. This is because group I extensor input can affect the amplitude of the extensor burst by at least two other reflex pathways in addition to the oligosynaptic pathway discussed above. The first pathway is the monosynaptic pathway from extensor group Ia afferents to extensor motoneurons. Stimulation of extensor group Ia afferents strongly excites mainly the ankle extensor motoneurons (Eccles et al., 1957). The second identified pathway is disynaptic and globally excites extensor motoneurons (Angel et al., 1996).

Verification of whether the *amplitude* of the ankle extensor activity increases following stimulation of the MG group I afferents in a cat with a previously axotomized LGS nerve awaits the conclusion of experiments that adopt either of these approaches: (1) in a spontaneously walking cat, the MG group I afferents could be stimulated and the resultant increases in LGS

ENG could be recorded or (2) intracellular recordings could be made in the MG motoneurons on each side of the cord and the effects of stimulating the group I afferents in this nerve on the amplitude of the monosynaptic, disynaptic and slow rising EPSP could be measured.

In the intact cat it is clear the the amplitude of the MG EMG during stance increases immediately following axotomy of the LGS nerve. This initial increase in the amplitude of the extensor EMG amplitude may be caused by increased group Ia and Ib afferent input from the MG muscle onto monosynaptic and polysynaptic reflex pathways which excite the MG motoneuronal pool and is likely not due to plastic changes. However, it is clear that this increase in MG EMG amplitude cannot prevent the increased yield of the ankle during the E_2 portion of stance from occuring. The increased group I feedback from the MG muscle may form an error signal which could lead to the eventual recalibration of the MG group I pathway. One way to test this possibility would be to immobilize the ankle after cutting the LGS nerve and seeing if the increase in the effectiveness of stimulating the MG group I pathway could be abolished. While our data show that a reduction in the yield of the ankle was greatest in the animal that exhibited the greatest increase in the effectiveness of the MG group I pathway, it is clear that a greater amount of data need to be collected before any correlation with a reduction in the yield of the limb can be considered.

Indeed, other mechanisms other than changes in reflex pathways may also contribute to the reduction in the yield of the ankle. For example, plastic changes in the extensor muscles occur after functional overload and may include changes in the oxidative capacity of the muscle, hypertrophy, and changes in fibre type (Edgerton et al., 1993). Since a progressive hypertrophy of the MG muscle occurred, it is likely that plastic changes in the muscle contributed to the recovery of the yield of the ankle (Edgerton et al., 1993). In all likelihood the initial response to

the injury is mediated by cortical structures, followed by long-term plastic changes in both the muscle and the extensor reflex pathways

Time course of the changes in the strength of the LGS and MG group I pathway

In chapter 4 we showed that while the plasticity of the locomotor dependent group I pathway regulating stance showed some similarities with plasticity of the group Ia pathway, one important difference was that the plasticity of the oligosynaptic group I pathway occurred relatively rapidly. One question that arose from that study was whether changes could occur faster than three days. In this chapter we have demonstrated that a progressive reduction in the strength of the LGS oligosynaptic group I pathway occurs over time after axotomy of the LGS nerve. These decreases in the strength of LGS group I stimulation become apparent 3 days (but not at day 2) after axotomy of the LGS nerve and showed a rapid decline over the ensuing days. This is in contrast to studies which have examined the plasticity that occurs in the group Ia monosynaptic pathway following similar axotomies (Eccles et al., 1959; Eccles et al., 1962). While decreases in the amplitude of the EPSP or the monosynaptic reflex occurred, these effects were not apparent until at least one week following the axotomy of the LGS or MG nerve (Eccles et al., 1959; Eccles et al., 1953; Gallego et al., 1979).

Stimulation of the MG group I afferents in the experimental leg produced increased effects on the duration of the extensor burst after only 3 days following axotomy of the LGS nerve. However, the data were quite scattered and no trend for a progressive increase could be observed. Increases in the amplitude of synergistic monosynaptic reflexes has also been reported to occur following axotomy of the heteronymous nerve (Eccles et al., 1962). However, these increases in the amplitude of monosynaptic excitation of synergists were challenged by Gallego et al. (1979) who failed to observe any increase. One issue is why did we fail to see a progressive increase in the effects of stimulating the MG group I afferents? One possible explanation is that the plasticity

of the MG group I pathway may be dependent on the activity level of the animal. From casual observations we noted that the activity level of each of the animals varied considerably. If the plastic effects on the MG group I pathway are activity-dependent then this may explain the large variability in the effectiveness of stimulation of the MG group I afferents from animal to animal.

The long-term changes in the strength of the LGS and MG group I pathways were affected by transection of the spinal cord

One of the questions asked in this study was whether the locus of plasticity was located in the spinal cord. After spinalization stimulation of the previously axotomized group I afferents in the LGS nerve still had a reduced effect on the duration of stance. Therefore, the site for the plasticity causing the reduction in the effectiveness of the LGS group I pathway was located within the spinal cord. In studies which have assessed the plasticity of the monosynaptic group Ia pathway after axotomy, the reductions in the amplitude of the EPSP are evident in the spinalized animal (Gallego et al., 1979). Therefore, the reduction in the strength of the LGS group I pathway is not dependent on descending inputs and is probably due to changes that are a direct result of axotomy of the nerve (e.g. retraction of the axonal synapses, altered postsynaptic receptor densities etc; see Mendell for review (1984)).

The results for the changes in effectiveness of the MG group I pathway are more difficult to interpret. While in the decerebrate state the effects of stimulating the experimental MG group I afferents had a greater effect on the duration of the extensor burst these differences were not always conserved following transection of the spinal cord. These results suggest that supraspinal tracts contribute to the increase in effectiveness of the MG group I pathway. Several behavioral studies indicate that adaptive changes in gait following injury is seriously impaired after supraspinal input is removed (Goldberger, 1977, 1988; S. Rossignol, Personal Communication). In the monkey it has been shown that supraspinal influences alter the expression of operantly

conditioned monosynaptic reflexes (Wolpaw and Carp, 1993). Since descending supraspinal inputs helped maintain the plasticity of the MG group I pathway in some of our animals it is tempting to suggest that this is one mechanism by which reflex pathways are optimized depending on the environmental conditions. However, this interpretation is challenged by data from 2 of our animals which show that the increased effects from stimulation of the MG group I afferents in the decerebrate state are maintained following spinalization of the cat.

An important question is whether a transected spinal cord of a cat could learn to recalibrate the MG group I pathway following axotomy of the LGS nerve. In the future we propose to test this idea by training spinal animals to walk and then axotomizing the LGS nerve. Our hypothesis is that the animals would not be able to recover following this deficit and, moreover, the recalibration of their group I excitatory pathway would not occur. Even if plasticity of the MG group I pathway *did* develop in the chronic spinal cat, it would not necessarily mean that the supraspinal mechanisms are not involved, since different mechanisms may mediate the plasticity in the intact animal.

In conclusion the experiments reported in this chapter have established that long-term changes in the strength of a reflex pathway that can control the amplitude and duration of the extensor burst occurs in cats when a extensor nerve is axotomized. The group I reflex pathway is recalibrated within 3-5 days so that afferent input from the remaining synergist muscles has an increased effect on the duration of the step cycle. In the future it will be important to establish whether the effects of stimulating group I extensor afferents in MG can have an increased effect on increasing the amplitude of the MG extensor burst following axotomy of the LGS nerve. It this did occur it would support our hypothesis that a reduction in the yield of the ankle could be partly accounted for by an increase in the strength of the MG group I pathways.

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Chapter 6

General Discussion

In this thesis we have demonstrated that activation of group I extensor afferents can control the duration and magnitude of the extensor burst in walking cats. We have also demonstrated that the effects of group I extensor afferent stimulation can be modulated in a task-dependent manner in the intact cat and can be recalibrated after axotomy of extensor nerves. In this chapter we will group together the main findings of this thesis from all the chapters in three main areas: (1) the afferent control of the stance to swing transition, (2) the afferent effects on the amplitude of the extensor burst, and (3) the plasticity of the extensor group I pathway. The final section is devoted to updating some established reflex pathways and suggesting how they may interact with supraspinal inputs.

Summary of main findings

The control of the stance to swing transition

Chapters 2 and 3 of this thesis present results which show that stimulation of afferents which innervate primary endings of the muscle spindles and the GTOs can functionally regulate the stance phase in walking cats. These responses were most evident in the decerebrate walking cat in which stimulation of these group I afferents often prolong stance for the duration of the stimulus train and delay the onset of the next flexor burst. These findings were predicted in previous investigations which had shown that proprioceptive input could affect the timing of the locomotor rhythm in fictively locomoting preparations (Conway et al., 1987; Guertin et al., 1995a) or in animals with greatly reduced afferent input (Pearson et al., 1992; Pearson and Collins, 1993). In chapter 3 we reported that qualitatively similar responses could be observed in

the intact walking animal. While the effects were qualitatively similar, there were important differences. Firstly, the effects on the duration of stance were less than what we had expected based on the findings using decerebrate cats (chapter 2) and secondly, the effects were very variable. Part of the reason for the effects being less than expected was due to plasticity of the group I LGS pathway that occurred after axotomy of the nerve in the intact cat (see chapter 4 & 5). However, the reduction in efficacy was also due to the behavioral state of the intact cat, as decerebration of these animals increased the effects.

An important feature of our experiments was that the effects of stimulating the LGS group I afferents depended on the task that the animal was performing. When the animal was walking bipedally, stimulation of extensor group I afferents had a greater effect on the duration of stance than when the animal was walking quadrupedally. It is interesting that in chapter 5, we saw that following axotomy of the LGS nerve, animals suffered greater deficits during quadrupedal than bipedal walking. In this regard, Goldberger (1988) noticed that after unilateral deafferentation, while animals could recover the ability to walk quadrupedally after 2-3 weeks they could never recover the ability to bipedally walk. Goldberger (1988a) came to the conclusion that during bipedal walking the animal relied on afferent input to a greater degree than during quadrupedal stepping. Our data tend to support this assertion, since the effects of group I stimulation increased during bipedal stepping.

In the future it will be interesting to see if the effects of group I extensor feedback on the timing of the step cycle alters when performing locomotor tasks that demand precise placing of the limbs. Goldberger (1977) for example, found that cats with unilateral deafferentation could not perform walking tasks that required accurate placing of the limbs. However, animals with just

one dorsal root spared could perform this task (Goldberger, 1988b). These data strongly suggest that some form of afferent feedback is necessary to perform these complex tasks.

Afferent effects on the amplitude of the extensor burst.

In locomoting spinal cats, stimulation of group I extensor afferents has a large effect on the amplitude of the extensor burst. Evidence suggests that both extensor group Ia and Ib afferents project onto an oligosynaptic reflex pathway that is only open during locomotion and which accesses the extensor half-centre to globally excite the extensors (Guertin et al., 1995a; Pearson and Collins, 1993; Gossard et al., 1994). It has been shown that an additional disynaptic excitatory pathway from group I extensor afferents which was closed in the spinal animal is open in the fictively locomoting decerebrate cat. The current view is that afferent inputs to the oligosynaptic, disynaptic and monosynaptic group Ia pathways act to reinforce the activity of the extensor burst during locomotion (Guertin et al., 1995). In the fictively locomoting cat, the effects of stimulating the ankle extensor afferents had the largest effects on the amplitude of ankle extensor ENG's suggesting that a global excitation does not occur (Guertin et al., 1995a). In both the intact (chapter 3) and decerebrate (chapter 2) walking cat we found that the excitation of extensor muscles was not evenly distributed following stimulation of extensor group I afferents. Similar to the findings of Guertin et al. (1995a) the main effects on the amplitude were distributed to close synergist muscles.

Contrary to our predictions, stimulation of the plantaris nerve (there are no monosynaptic Ia connections onto the MG muscle) did not increase the EMG amplitude in the MG. Instead, any effects on the amplitude of the extensor bursts were mostly observed during late stance. These results contradicted reports showing a powerful effect of stimulating these afferents on the amplitude of the MG extensor burst in spinal (Pearson and Collins, 1993) or decerebrate cats (Guertin et al., 1995a). One possibility that may explain these differences is the extensor half-

centre is more active in both the intact and decerebrate walking cat compared to the locomoting spinal preparation. In support of this idea Gossard et al. (1994) found that the amplitude of the slow EPSP, produced by activation of the oligosynaptic pathway, was most attenuated during the middle of the extensor ENG burst, presumably when the extensor half-centre is most active. Since in walking animals we would expect the extensor half-centre to be activated to a greater extent than in paralyzed spinal animals, presumably occlusion of extensor group I inputs onto the extensor half-centre would also be greater.

While the gain of the oligosynaptic group I afferent pathway appears to be low (PL onto MG for example) in walking animals, this may be a consequence of the artificial nature of stimulating single extensor nerves. Normally when a limb is loaded extensor group I afferents from many different muscles are activated. When different extensor nerves were stimulated simultaneously at group I strengths we noticed that the effects on the duration (chapter 2) *and* amplitude (chapter 3 and appendix 2) of the extensor bursts increased. Functionally, the oligosynaptic pathway may work optimally to control the amplitude when many extensor muscles are loaded and active simultaneously. While attractive there are a few holes in this theory. Stimulation of the lateral gastrocnemius and soleus (LGS) nerve (actually stimulation of two individual nerves LG and S), has a powerful affect on the amplitude of the medial gastrocnemius (MG) EMG nerve but has virtually no effect on the amplitude of the vastus lateralis (VL) EMG (chapter 3). Since the oligosynaptic group I pathway is thought to excite the extensor half-centre (Conway et al., 1987; Gossard et al., 1994; Pearson and Collins, 1993; Pearson et al., 1992), one would expect equal activation of all extensor muscles. One possibility is that the oligosynaptic pathway may be involved more in affecting the *timing* rather than the amplitude of the extensor burst and that other excitatory pathway control the *amplitude*. Our data support this proposal, as stimulation of group I extensor afferents were more likely to affect the duration of the extensor burst rather than

reinforce its amplitude. It is still not clear how the large localized increases in the amplitude of the MG could occur. Since disynaptic (Angel et al., 1994; Guertin et al., 1994) and oligosynaptic extensor group I pathways both generally excite extensors, the logical conclusion is that monosynaptic connections (which are localized Eccles et al., 1957b) between the triceps surae are responsible for these effects. Yet, the gain of the monosynaptic heteronymous reflex is low in the intact and decerebrate cat (Personal observations; Duenas et al., 1990). McCrea et al., (1995b) has reported in a recent abstract that 'plateau like potentials' occur in ankle extensor motoneurons when ankle group I afferents are stimulated suggesting some type of complex interaction between the three excitatory pathways. It is clear that we must wait on a detailed intracellular analysis to find out the relative contributions of the monosynaptic, disynaptic and oligosynaptic pathways to the amplitude of the extensor burst.

To summarize when an individual extensor muscle is perturbed, we would expect from our results that the effects on the amplitude of the extensor burst would be localized to close synergists. This would be useful since it would act to correct the perturbation around a single joint, without large effects on the timing of the step cycle. As the group I afferents from neighboring extensor muscles are coactivated we would expect that the oligosynaptic pathway would begin to affect the duration of the extensor burst. A complex interaction between the oligosynaptic, monosynaptic and disynaptic pathways likely contributes to the localized increase in amplitude in intact animals.

Plasticity of the group I pathway

Animals are in a constant state of flux as they develop, age, and react to environmental pressures. To successfully adapt to these changes, neural networks have to be fine-tuned to function optimally. One of the most interesting findings on the function of group I extensor feedback during locomotion is that the reflex pathways can be recalibrated upon injury to an extensor

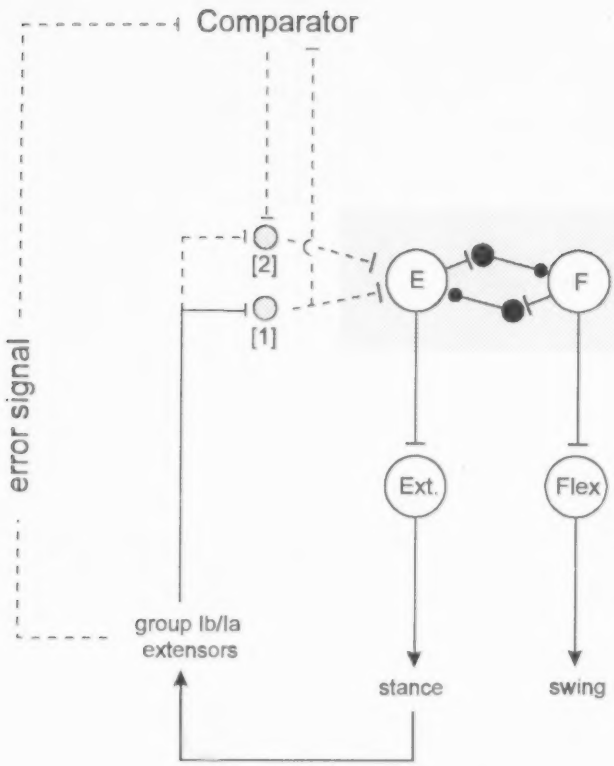

Figure 6-0: A schematic proposing how parallel pathways may increase the influence of the MG group I pathway. Normally the group I extensor afferents from the MG muscle excite the extensor half-centre by way of a locomotor dependent oligosynaptic pathway (1). When the LGS nerve is axotomized, the load carried by the MG muscle increases and an increased yield of the ankle occurs during the E_2 phase of stance. Presumably, the increased load of the MG muscle and the increased yield of the ankle would lead to increased activity of the group Ib and Ia afferents respectively. This error signal would then be compared to the activity of the MG group I oligosynaptic pathway. The comparator would increase the effectiveness of the MG group I pathway by opening a parallel pathway located in the spinal cord (2).

nerve (chapter 4 & 5). Within days the effectiveness of stimulating the cut LGS nerve (at group I stimulus strengths) on prolonging stance was reduced compared to stimulation of a freshly axotomized LGS nerve. In addition, the effects on the amplitude of the MG extensor burst were also reduced (in animals in which the MG nerve was not cut). While we reported these findings extensively in chapter 4 and 5, it is clear that these results impinge on the results from chapter 3. In intact animals in which we stimulated the LGS nerve, the effects on the duration and the amplitude of the MG extensor burst declined from day to day. Since the amplitude of the monosynaptic reflex did not change this indicates that these declines were mainly due to changes in the oligosynaptic and possibly disynaptic pathways (since amplitude was affected) pathways. This result raises a warning flag, for since plasticity of the extensor group I pathway can occur within 3-5 days it should make us very cautious when interpreting experiments in which axotomized nerves are used to assess reflex function in intact animals.

In chapter 4 & 5 we showed that stimulation of MG group I afferents in the leg containing the previously cut LGS nerve had an increased effect on the duration of stance compared to the control leg. Thus, within a short period of time, recalibration of this group I reflex pathway allows the weighting of sensory input from the only functioning member of the ankle extensors to be dramatically increased. Unfortunately, we were not able to conclude that the amplitude of the extensor burst was increased in other members of the triceps surae following stimulation of the MG group I afferents since they were denervated. However, the results for LGS mentioned above as well as results presented in appendix 2 of this thesis suggest that increases in duration are accompanied by increases in the amplitude of the bursts in synergistic extensor muscles. Therefore, we suggest that the effects of stimulating the MG group I afferents also has a greater effect on the amplitude of the extensor burst following axotomy.

In chapter 5, we observed that while the decreased effectiveness of the LGS group I pathway was conserved, the increased effectiveness of the MG group I pathway was not always observed following spinalization. This finding supports the idea that supraspinal descending influences led to the maintenance and induction of the plasticity in the MG pathway. In figure 6-0, I have outlined one possible network that could lead to the recalibration of the MG group I pathway. The template for this pathway was obtained from models proposed by Lisberger (1988) to explain the recalibration of the vestibuloocular reflex. An important point to emphasize is that this pathway is entirely speculative and other solutions are possible. Compared to normal locomotion, when the LGS nerve is cut, the following changes occur in the intact cat during stance: (1) there is a greater yield of the ankle and, (2) there is a large increase in the amplitude of the MG EMG. This would presumably result in a greater afferent ensemble firing rate from ankle extensor group Ia (increased yield) and group Ib afferents (increased load) during early stance (Prochazka et al., 1989). This increased sensory feedback can be thought of as an error signal informing the CNS that it needs to increase its locomotor output. It is known that dorsal spinocerebellar tract (DSCT) neurons respond preferentially to activity in extensor muscles (Laporte et al., 1956; Lundberg and Oscarsson, 1956; Lindstrom and Takata, 1972; Walmsley, 1992; Arshavsky et al., 1972b; 1986). The DSCT, which ascends to the cerebellum, may therefore carry the required error signal for recalibration of the MG group I pathway. It has been suggested by Lundberg et al. (1971) that the cerebellum compares the output of the pattern generator to the sensory feedback produced by the DSCT. The output of the pattern generator is thought to be signaled by neurons ascending in the ventral spinocerebellartract (VSCT) (Arshavsky et al., 1972 a,b,d; 1986; Lundberg, 1971). The output of the cerebellum could result in the appropriate corrective signal being generated to increase the activity of the MG group I pathway by opening parallel pathways as shown in figure 6-0.

Before attempting to formulate hypotheses to test this proposal it should be demonstrated that the isolated spinal cord cannot learn to recalibrate the MG group I pathway upon axotomy of the LGS nerve. To test this hypothesis it would be necessary to train cats with a transected spinal cord to walk and then cut the LGS nerve. After a week or so the MG and LGS nerves would be stimulated in each leg during stance to see if any plasticity had occurred. If as predicted no changes in the MG group I pathway occur in the spinal animals then one could conclude that supraspinal inputs were necessary to induce the plasticity. A positive result does not on the other hand rule out a role for supraspinal inputs as both spinal and supraspinal processes could contribute to the plasticity (cf Wolpaw and Carp, 1993).

We have presented no data on the anatomical mechanisms that may contribute to the long-term changes in the strength of the LGS or MG group I pathways. Since the effects are on the duration and not the amplitude of the extensor burst, this suggests that the connections from group I afferents onto interneurons comprising the extensor half-centre are altered. Gossard et al. (1994) have recorded candidate interneurons located in lamina VII which may form part of this interneuronal network. This is consistent with anatomical studies which have shown using horseradish peroxidase widespread terminal arborizations from both group Ia and Ib afferents in lamina VII (see Brown, 1981 for review). Hopefully, in the future these interneurons will be positively identified which would allow a greater understanding of whether post or presynaptic mechanisms are responsible for the changes in the strength of the LGS or MG group I pathways observed after axotomy.

To summarize, our results suggest that the strength of particular extensor group I pathways are flexibly adjusted to match the afferent inflow from a particular extensor muscle. There are many uses for a system such as this. Development, injury, athletic training, space flight, and pregnancy

are all examples of situations when the load carried by our extensor muscles changes over a relatively long period of time.

Possible reflex pathways for the control of locomotion

The spinal pathway

A scheme summarizing the inputs from the proprioceptive and exteroceptive afferents is shown in fig. 6-1. This model incorporates the idea that the locomotor rhythm is generated by mutually inhibiting half-centres (Lundberg, 1981; Jankowska et al., 1967a,b). One of the problems with the half-centre model initially proposed by Lundberg (1969) was that it predicted a simple alternation between flexors and extensors during walking. The complex activity of flexors and extensors that is observed during walking (Engberg and Lundberg, 1969) was initially hypothesized to be due to afferent feedback which sculpted the simple rhythm produced by the half-centre (Lundberg, 1969). This proposal was contradicted by experimental data obtained from postmammillary decerebrate cats that were walking on the treadmill which clearly showed that a complex pattern of muscle activity could be produced without afferent input (Grillner and Zangger, 1984). In light of this, the half-centre model has been expanded by Perret (1983) to allow for a central oscillatory network (half-centre model) that produces the general timing of the network and a system of premotoneuronal interneurons which sculpts the final output and produces the complex activation of extensor and flexor muscles that is observed during stepping. Similar proposals have been made for the operation of the scratch reflex (Koshland and Smith, 1989), and for the respiratory system (Feldman, 1983). As a result, the half-centre hypothesis remains an appropriate model for discussing the actions of afferent feedback on the timing of the locomotor pattern. For the sake of simplicity the premotoneuronal network proposed by Perret (1983) is not indicated in figure 6-1. In this model it is assumed that both extensor and flexor afferents project onto the extensor half-centre and affect the timing and the amplitude of the

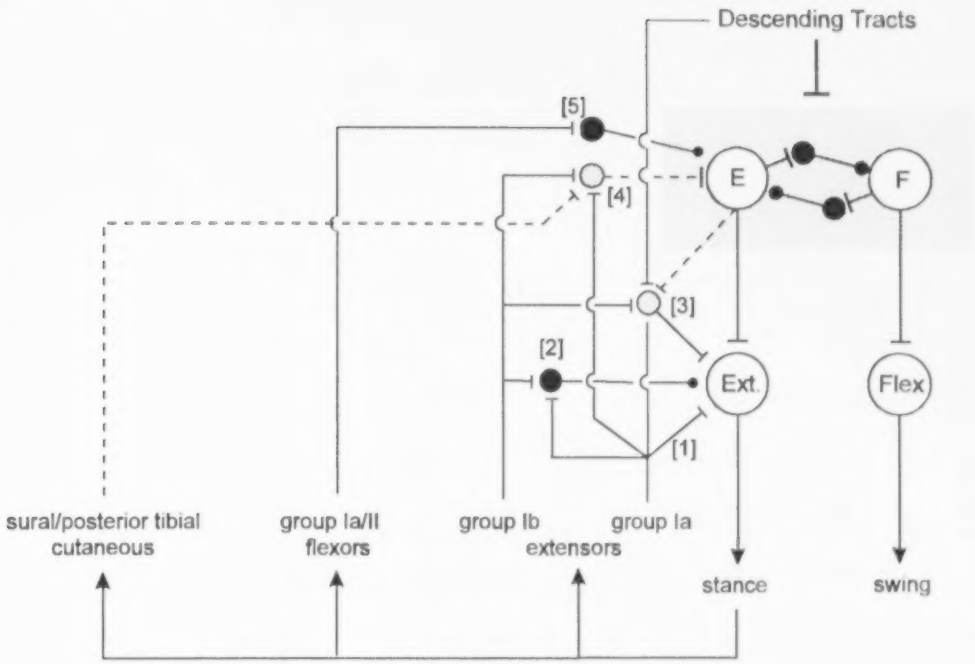

Figure 6-1: Pathways indicating how afferents from proprioceptor and cutaneous receptors can affect the extensor burst activity. The shaded box represents the CPG that generates locomotion via the mutually inhibiting extensor and flexor half-centres (as proposed by Lundberg, 1981). During locomotion, the normally inhibitory pathway from the group Ib and group Ia afferents (2) is inhibited and an oligosynaptic pathway opens (4) which excites the extensor half-centre when group Ib and Ia afferents are active during stance. In addition to the excitatory input from the extensors, the extensor half-centre also receives inhibitory feedback (5) from the flexor muscle afferents which are active during late stance. A disynaptic excitatory input from group Ia and group Ib afferents is also open during locomotion (3) which has been documented only during MLR evoked locomotion. Cutaneous input (as indicated by the dotted line) may also contribute to the excitation of the extensor half-centre but this has not been established conclusively. Parts of this circuit diagram were published by Pearson (1996). Modified with permission from Pearson (1996).

extensor burst (pathway 4 in figure 6-1). Afferent input from group I extensor afferents may also affect the amplitude of the extensor burst by acting on the premotoneuronal network that lies outside the CPG (McCrea et al., 1995b; pathway 3 in figure 6-1).

During locomotion group I afferent feedback from the extensors causes both an excitation of the extensor burst and an alteration in the timing of the step cycle. Due to the long latency of the EPSP in extensor motoneurons when group I extensor afferents are stimulated, the pathway is likely polysynaptic. This pathway receives convergent afferent information from many different extensor muscles (chapter 2 & 3; however see Gossard et al. 1994) and globally excites the extensor muscles in the ipsilateral limb by an excitatory action on the extensor half-centre (Guertin et al., 1995a). Group Ia feedback from muscle spindle afferents also excites the extensor half-centre (Guertin et al., 1995a) as well as directly exciting extensor motoneurons (pathways 1, 3, & 4 in figure 6-1). Thus, combined activity from group Ia and Ib afferents located in extensor muscles tends to prolong the extensor burst and prevent premature flexion of the limb while the limb is loaded. When extensor group I afferents from more than one nerve are stimulated at once the effects on duration are often greater than when they are stimulated in isolation (chapter 2, appendix 2). It is possible that parallel pathways from group I extensor afferents exist that project onto separate pools of interneurons before projecting onto the extensor half-centre may explain this phenomenon.

Extensor group I afferents are not the only afferent signals which can control the step cycle. The flexor muscle spindle afferents inhibit the extensor half-centre (pathway 5 in figure 6-1) and thus act to curtail the extensor burst and cause the onset of the swing phase (Hiebert et al., 1996). In theory, there are two possibilities by which the flexor muscle afferents could affect the timing of the step cycle: (1) The flexor muscle afferents could directly activate the flexor half-centre and

inhibit extensor activity by the resultant inhibition of the extensor half-centre or (2) The flexor afferents could activate the flexor half-centre by inhibiting the activity of the active extensor half-centre. Although both are plausible, evidence favors the latter possibility. This is based on two pieces of evidence: (1) the latency for inhibition to occur in the extensor muscles is extremely rapid and is similar to that reported for extensor afferents while in contrast, (2) the minimum latency for the initiation of flexor activity was 90 ms, indicating that the extensor half-centre is inhibited before the flexor half-centre (Hiebert et al., 1996).

The stance to swing transition is likely initiated by a combination of a reduction in extensor afferent feedback due to the unloading of the limb combined with an inhibition of the extensor half-centre by flexor muscle afferents as the leg is extended and the flexor muscles are stretched. However, it is clear that during stimulation of the LGS nerve the leg is powerfully extended at the hip, knee and especially the ankle (chapter 2). In this case it is clear that signals from the flexor muscle afferents that are stretched can not overcome this powerful extension. This may be functionally relevant as the maintenance of ground support is critical when extensor muscles are loaded and under these conditions feedback from flexor muscle afferents may be less effective (see Hiebert, 1996). A contribution from certain low-threshold cutaneous afferents such as those within the sural nerve (Duysens and Pearson, 1976; Duysens, 1977; Duysens and Stein, 1978; Guertin et al., 1995) likely contribute to the excitation of the extensor half-centre, as indicated by the dotted line in figure 6-1.

While it is assumed in this model for the sake of simplicity that many of the effects on the step cycle from groups of afferents excite or inhibit interneurons that comprise the extensor half-centre it is entirely possible that these afferents may also project onto the flexor half-centre. For example in fictively locomoting decerebrate cats, resetting of the locomotor rhythm by

stimulation of the group I extensor afferents during the flexor phase of the locomotor cycle is accompanied by a simultaneous excitation of the extensor motoneurons and by an inhibition of the flexor motoneurons (Guertin et al., 1995b). These results suggest a more global regulation of the central pattern generator, rather than a selective input to the extensor half-centre.

Supraspinal control of reflex pathways

Descending inputs onto the simplified network shown above (figure 6-1) can modify the locomotor output (Armstrong, 1986; Rossignol, 1996). At present our knowledge of this control is sparse. Some of the pathways which may control the expression of the extensor group I excitatory pathways are summarized in figure 6-2. We have no information on the supraspinal control of the flexor afferent system. Firstly, in the decerebrate cat (chapter 3) we found that stimulating the MLR at increasing stimulus intensity levels resulted in a decrease in the effectiveness of stimulating the group I extensor afferents. We know that the pathway from the MLR travels through the medullary reticular formation and onwards via the ventrolateral funiculus to the rhythm generating centres of the spinal cord (Jordan, 1991). Noga et al. (1995) have observed that stimulation of the cuneiform nucleus (a component of the MLR) can lead to a reduction in the group I field potentials at latencies that are consistent with activation of fast reticulospinal pathways. These MLR effects may be exerted pre or postsynaptically. Therefore, I have included both pathways in figure 6-2. These findings must also be viewed in light of the fact that stimulation of the MLR has been shown to open the oligosynaptic and disynaptic group I pathway in fictively locomoting animals (Gossard et al., 1994; McCrea et al., 1995). For example, it could be possible that the descending signals from the MLR may have open the group I oligosynaptic pathway and concomitantly increase the level of presynaptic inhibition onto the group I afferents.

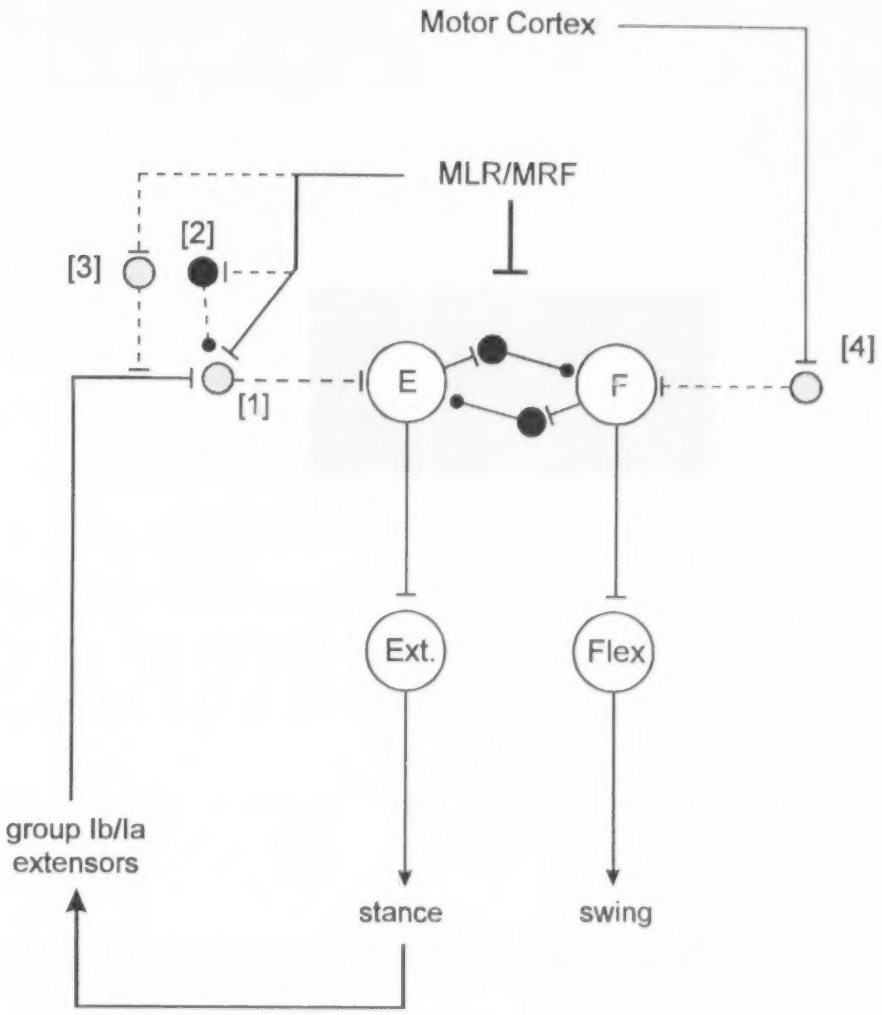

Figure 6-2: A schematic showing the influence of descending projections on the control of the extensor group I pathways. When locomotion commences descending signals from the MLR open up a oligosynaptic pathway from group I extensor afferents (1). When the intensity of the stimulus to the MLR is increased an inhibitory pathway may be recruited that reduces the effectiveness of the group I oligosynaptic pathway (2). Alternatively, activation of descending pathways from the MLR could increase the level of presynaptic inhibition onto the group I extensor afferents, thereby reducing synaptic transmission. Activity of the motor cortex is known to affect the transition from stance to swing, by inh biting stance. Since stimulation of cortical structures also promotes flexion, it likely excites the flexor half-centre (4).

The motor cortex may also control the expression of the extensor group I pathway. In chapter 3 we showed that in the intact cat, stimulation of group I afferents had variable effects on the duration of stance. In addition the pathway was modulated depending on the task. Investigations of the role of the motor cortex demonstrate that microstimulation of cortical cells can cause a premature transition from stance to swing, much like the effects of stimulating the flexor group I afferents (Drew, 1991). Recordings from cortical cells during normal locomotion show that cells are most active at the transition from stance to swing (Drew, 1991). These results suggest that the motor cortex excites the flexor half-centre and would be expected to potentiate the effects of the flexor group I afferents and suppress the effects of extensor group I afferents around the time of the stance to swing transition. Since the cells of the motor cortex are most active during the performance of complex locomotor tasks (Drew, 1991, 1988; Beloozerova and Sirota, 1993; 1988), it is tempting to suggest that the importance of the extensor group I afferents wane during the performance of complex tasks.

Closing Statements

It is evident that sensory feedback plays an invaluable role in adjusting the basic locomotor pattern to the vigors and complexities of an ever changing environment. For example a chronic spinal cat can adjust its cadence to the speed of a moving treadmill by relying solely on sensory feedback from the moving limbs. It has been shown that afferent feedback can alter the timing of the step cycle in cats by changing the length of the extensor burst. The first part of my thesis work explored the effects of stimulation of extensor group I afferents (relaying input from muscle spindles and golgi tendon organs) on the stance phase in walking cats. We discovered that stimulation of the group I afferents operating through a previously proposed oligosynaptic pathway could powerfully extend the stance phase and delay flexion in decerebrate cats. Similar but less robust effects were observed when comparable stimuli were applied to intact cats. One

major question that arose was what factors could contribute to these differences between the stimulation of group I afferents in intact and decerebrate cats. We found from further work in the decerebrate walking cat that under certain conditions the effectiveness of the strong peripheral input from group I afferents could be modulated. In particular, increased stimulation of locomotor centres in the brainstem could decrease the efficacy of the group I stimulation. In addition to these studies we explored the possibility of plasticity in the locomotor-dependent group I excitatory pathway. We found that three to four days after cutting the LGS nerve in an otherwise intact cat stimulation of the synergistic medial gastrocnemius (MG) group I afferents would increase the duration of the extensor burst to a greater extent than similar stimulation of the contralateral MG nerve. Interestingly, the recovery of function in the intact cat after axotomy appeared to be correlated to an increase in the efficacy of the MG afferents. The recalibration of the MG group I pathway was influenced by supraspinal sites as transection of the spinal cord abolished the plasticity in many animals. In summary, my thesis work has shown that extensor afferent feedback can affect the timing and the amplitude of the extensor burst. These effects are dependent on the state of the animal, and the central pathways mediating this effect can be recalibrated in response to peripheral injury.

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Appendix 1

Stimulation of the group I extensor afferents in the walking L-DOPA cat.

Conway et al. (1987) first described the effects of trains of extensor group I electrical stimuli on resetting and entraining the locomotor rhythm in L-DOPA cats. This study was followed by an intracellular analysis of the oligosynaptic extensor group I pathway opened after the administration of L-DOPA (Gossard et al., 1994). Both of these studies used fictively locomoting spinal animals which had been paralyzed using pancuronium. In the paper by Conway et al. (1987) stimulation of extensor group I afferents from different extensor muscles had similar effects on the amplitude and the duration of the extensor ENG burst. However, as pointed out by Gossard (1994) one disadvantage of the reduced preparation is that without phasic afferent feedback the excitability of the extensor half-centres was probably quite low, thus increasing the likelihood that stimulation of group I afferents could alter the timing of the step cycle. This appendix reports on the effects on stimulating the group I extensor afferents in a L-DOPA, acute spinal, cat that walked exceptionally well on a motorized treadmill. The rational for expanding on the results shown in chapter 5 are that these are the only studies which have examined the effects of stimulating group I extensor afferents in the walking L-DOPA animal. Also, L-DOPA animals were also used by Lundberg's group to provide evidence for mutually inhibiting half-centres participating in the generation of locomotion which are an important element of our theoretical models.

Methods

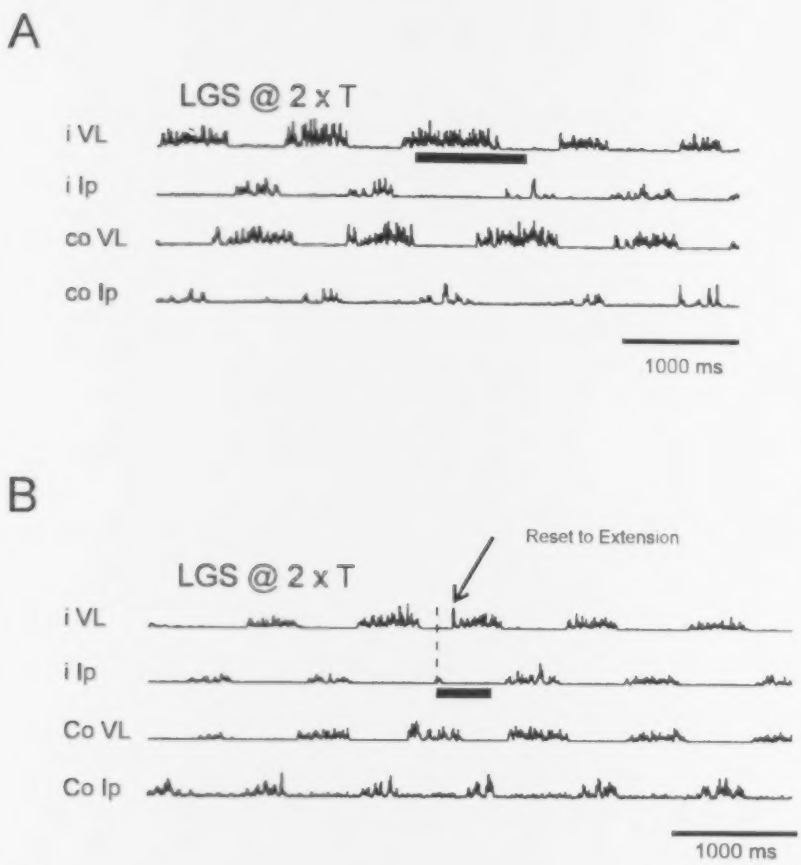
The full methods have been described in chapter 5 and only an abridged version will be described here. EMG electrodes (Cooner wire AS632) were implanted into the VL and IP muscles on the

cat in both hind legs. Stimulating electrodes were implanted onto the LGS and MG nerves on both legs and a sciatic nerve cuff was installed on both legs to monitor the stimulus volley and determine the stimulus threshold. The animal had its LGS nerve cut in a previous surgery 21 days before the acute procedure in the opposite limb. Only results from stimulating the 'normal' LGS nerve will be examined here.

The animal discussed in this section was decerebrated and spinalized at the T12 level. We then infused nialamide (50 mg/kg) dissolved in 20 ml of water into the animal over a period of 45 minutes. The nialamide (Sigma chemicals) was dissolved in an acidic medium and then buffered to a pH of approximately 3.5-4.5. After the nialamide had been administered, we infused methyl ester L-DOPA (50 mg/kg) (Sigma chemicals) dissolved in 5 ml of water. In approximately 20-30 minutes after the infusion of L-DOPA the animal began to step and continued to produce bouts of stepping for 3 hours.

Results and Discussion

In many of our L-DOPA cats the walking pattern produced was sporadic and consisted of shuffling movements of the hindlimbs. In the one animal reported here the walking pattern was extremely consistant and consisted of an identifiable F, E_1, E_2, and E_3 phase. This is characterized by the kinematics of a full step cycle presented in figure A1-0C. During these walking sequences perineal stimulation was applied and the animals weight was supported by holding the tail. The timing of the extensor and flexor bursts were equivalent to those obtained in the decerebrate state. The only deficit that we observed was during the flexor phase of the step cycle. The leg was not swung forward as much as in the intact or decerebrate cat.



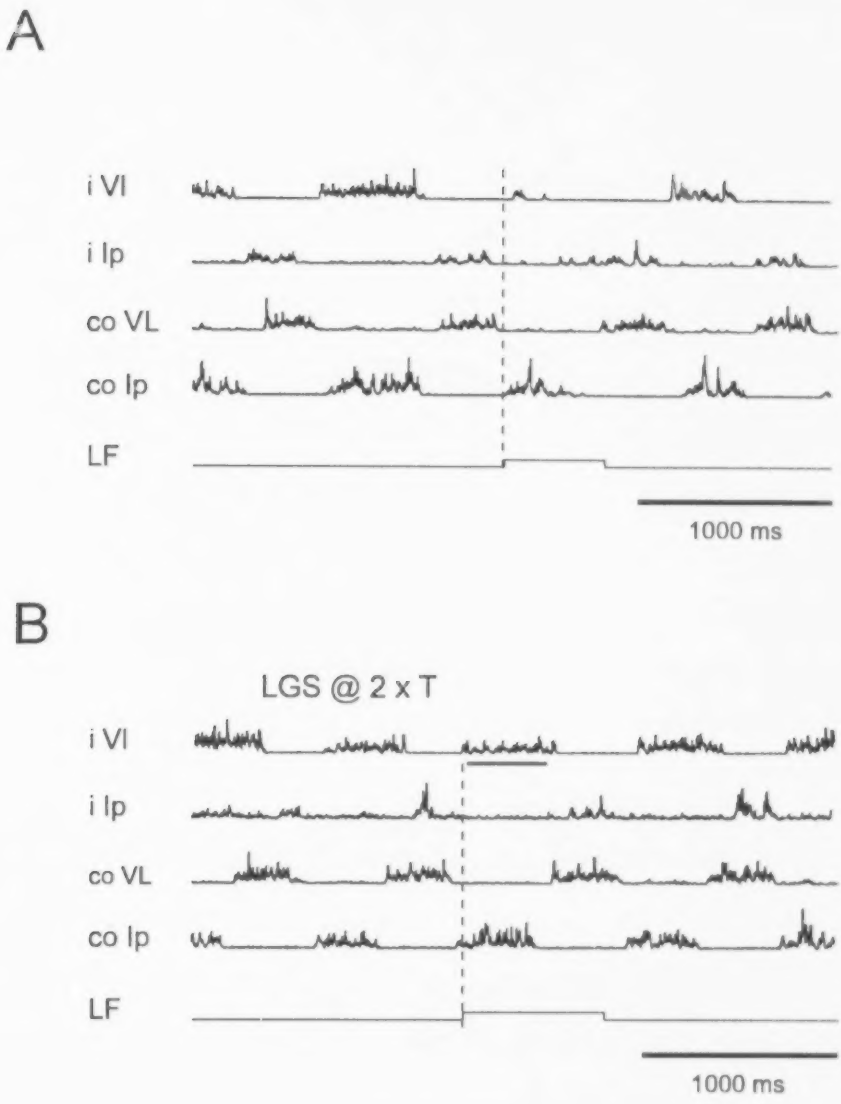
A1-0: Examples of the effects of stimulating the LGS group I afferents during extension and flexion in a L-DOPA treated stepping animal (spinal cord transected). A,B. Rectified and filtered EMG traces. A. Stimulation of the LGS group I afferents prolongs the duration of the extensor burst and delays flexion. B. Stimulation of the LGS group I afferents during flexion, terminated the ongoing flexor burst and reset the step cycle to extension. C. Kinematics of an unperturbed step cycle, showing the incomplete flexion of the leg, which was mainly a result of a incomplete flexion of the hip.

Effects of stimulating the extensor group I afferents during mid-stance.

Stimulation of the LGS or MG nerve at group I strengths had similar effects on the duration and the amplitude of the extensor bursts as we have shown in previous chapters using decerebrate animals. Stimulation of the LGS nerve often prolonged the duration of the extensor burst for close to the duration of the stimulus train (see figure A1-0A, c.f figure 5-5). In contrast stimulation of the MG nerve had a modest effect on the duration of the extensor burst (see figure 5-4). These results match our finding from decerebrate cats (chapter 2,3 and 5) which show that stimulation of the LGS nerve has a powerful effect on the duration of the step cycle. They are also similar to the results of Conway et al. (1987) who used fictively locomoting L-DOPA cats. Since the results were also very similar to those obtained in the decerebrate cat and qualitatively similar to the intact cat this suggests that the effects of group I afferent stimulation are conserved in different walking preparations.

Effects of stimulating extensor group I afferents during early flexion

Conway et al. (1987) showed that stimulating the extensor group I afferents during the early part of the flexor burst could inhibit the flexor burst and reset the step cycle to flexion. This result showed that stimulation of the group I afferents can have a large effect on the timing of the step cycle. In the walking DOPA treated spinal cat, stimulation of the LGS nerve also strongly inhibited the ongoing flexor burst and reset the ipsilateral VL burst to extension (figure A1-0B). These effects were visually apparent. The step cycle of the contralateral limb was also affected resulting in a full reset of the stepping rhythm. These effects were similar to those observed when the LGS nerve was stimulated during early flexion in decerebrate walking cats (chapter 2). In contrast, stimulation of the MG nerve had no effect on the timing of the step cycle when stimulus trains were delivered during mid-flexion.



A1-1: Stimulation of the extensor group I afferents can abolish the premature flexion of the foot of a L-DOPA walking cat when its foot entered a hole. A,B. Rectified and filtered EMG traces of a L-DOPA cat walking on a motorized treamill. A, When the foot entered the hole the extensor VL burst was abolished and a premature flexion response occurred. Dotted line indicates the onset of the light fence triggered by the foot entering the hole. B., When the stimulation of the LGS group I afferents were triggered off the onset of the light fence (dark line) the VL extensor burst was augmented and flexion of the leg was delayed.

Foot in Hole Trials

We also had an opportunity in this animal to test whether it would flex its foot out of a hole when ground contact was withdrawn. The treadmill belt was replaced with one which had a hole cut in it. At times the animal's leg would fall into the hole and trigger the light fence. As shown in figure A1-1A when the animal's leg fell into the hole its extensor burst was totally abolished and this triggered a premature flexion out of the hole. These limited results suggest that the classic foot-in-hole response observed in intact and chronic spinal cats (Gorassini et al., 1994; Hiebert et al., 1994) are also conserved in the L-DOPA animal. The results also match those of the decerebrate walking cat in which flexion out of a hole can also occur (Hiebert et al., 1995). Of special interest was the abolishing of the extensor burst in VL, this illustrates that part of the extensor burst must be accounted for by afferent feedback (see G.W. Hiebert, PhD Thesis). When the LGS and MG group I afferents were stimulated simultaneously when the leg went into the hole the amplitude of the extensor burst was increased and the leg was held in the hole for the duration of the stimulus train. These results are interesting for two reasons: (1) they agree with previous results obtained in the decerebrate walking cat and (2) the amplitude of the extensor burst was increased showing that the oligosynaptic pathway from LGS to VL can contribute to an increase in the amplitude of the VL EMG. An important caveat to these results using the foot in hole paradigm is that only one animal is presented and variations among animals could well occur. Therefore these results should be looked on as fodder for future experimentation.

Overall the results from this cat demonstrate the conservation of effects of stimulating the group I extensor afferents in different locomoting preparations. While the results from one walking L-DOPA animal is presented here, the results using other L-DOPA cats that did not walk as well did show similar results. This result when married with results obtained by K. Fouad and Keir Pearson (Personal Communication) in the walking rat suggest that the effects of extensor group I

afferent stimulation affects the walking pattern in similar ways both in different cat preparations and different mammalian species (Pearson, 1993). The same principles of afferent regulation of walking is remarkably similar to that proposed to function in the cockroach. The attractive hypothesis is that common principles of afferent regulation of walking exist throughout the animal kingdom (Pearson, 1993).

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Appendix 2

A collection of unpublished findings

In the course of every experiment you run across certain phenomena that for various reasons is left unpublished. I have decided in this short section to collate a number of our findings that *are* relevant to the thesis. Another reason for the existence of this appendix is a desire to leave the content of the papers alone and allow them to stand (chapter 2 and 4) as they were originally written. This section is referenced in the text a number of times and is not intended to stand alone. Therefore, without the appropriate contextual reference, it will (and is) feel a little disjointed. The first piece of information I wish to present is related to the issue of summation of effects upon stimulation of different extensor nerves. In chapter 2 we noted that stimulation of different extensor group I nerves could result in a non-linear increase in the prolonging of stance. Following publication of that paper, we repeated this joint stimulation of extensor nerves and subsequently realized that it was not just the duration of the extensor burst, it was also the amplitude. This is shown in figure A2-0. In this figure stimulation of either the PL or MG nerves in a spontaneously walking cat had a small effect on the duration or the amplitude of the LG extensor burst. But, when both were stimulated simultaneously both the effects on the amplitude and the duration of the extensor burst. One reason why this result is important is that it may have implications for the data in chapter 4 and 5 in which we know that the effectiveness of the MG group I pathway is increased but unfortunately we were not able to measure the amplitude since all its close synergists were cut.

The next result was collected using spontaneously walking decerebrate cats but relates mainly to our work on intact cats in chapter 3. At times we had noticed that the effects of stimulating the

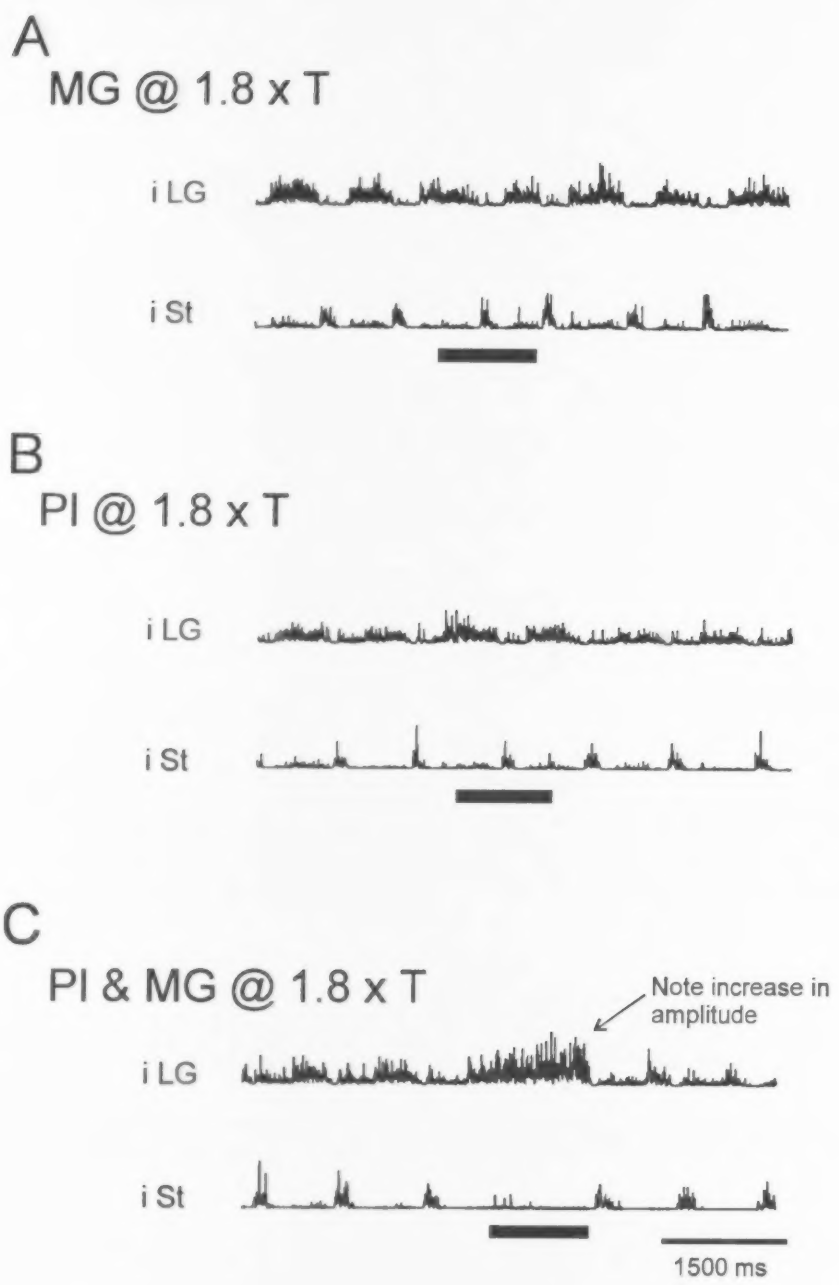

Figure A2-0: Stimulation of PL and MG group I afferents simultaneously results in a greater enhancement of the duration and amplitude of the extensor burst than when each are activated in isolation. A,B,C. Rectified and filtered EMG traces from a spontaneously walking decerebrate cat. Note that stimulation of the PL or MG group I afferents had small effects on the amplitude and duration of the LG EMG burst. However when both the MG and PL group i afferents were stimulated a large increase in the amplitude *and* duration of the extensor burst occurred.

extensor nerves while having more consistent effects than in the intact animal were also subject to variability. In one animal we were able to quantify that movement of the forelimbs affected the strength of the effects produced by stimulating the group I extensor afferents. Specifically, if the forelimbs stopped moving (figure A2-1A) stimulation of the extensor burst could prolong extension for the duration of the stimulus. In contrast the these effects were drastically reduced when the forelimbs began to step. Another feature that is nicely illustrated is the effects of the contralateral limb on the timing of the step cycle. Note in figure A2-1A that the stimulus offset occurred around the same time as the cat entered its contralateral swing phase. The ipsilateral leg 'waited' for the contralateral limb to finish swing before beginning its own swing. We found many further examples of this behavior after publication of chapter 2 and that is why it was not mentioned in that chapter. Interestingly, the opposite effect can be seen to the left of the dotted line in figure A2-1A, in this case one can see that the contralateral swing was delayed until the ipsilateral swing had finished. This evidence strongly suggests that there is a strong mutually inhibition of the flexor half-centres of each leg.

The next item to mention pertains to chapter 3 and relates to the issue of speed. We had anecdotally observed earlier that when the intact animal was 'striding forward' the effects appeared to be weaker than when the cat was ambling along. Figure A2-2 quantifies this relationship for a bipedally walking intact cat walking at several different speeds. As can be seen there was a trend for the effects of stimulating the LGS group I afferents to decrease as the speed of the treadmill increased.

The final section of this appendix covers some intracellular experiments we did on animals in which we had previously axotomized the LGS nerve. We began this series to see if we could obtain evidence for changes in the group I pathways that was so evident in the behavioral work

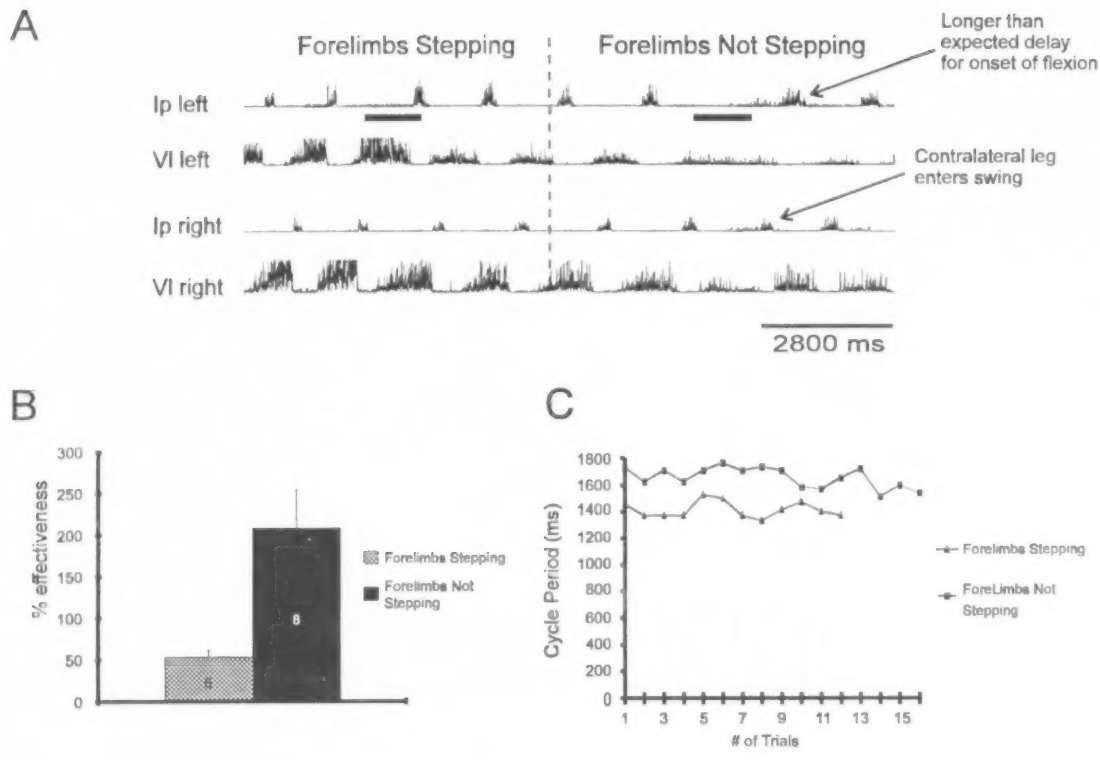

Figure A2-1: Stimulation of the group I extensor afferents in a spontaneously walking decerebrate cat prolonged stance for a greater period of time when the forelimbs were not moving. A. Rectified and filtered EMG traces which show the greater effects of stimulating the group I extensor afferents on prolonging the VL extensor burst when the forelimbs stopped moving (right side of the dotted line). B. Bar graph showing the percentage effectiveness of the stimulation of the MG group I afferents during periods when the forelimbs were not stepping (black bar) compared to periods when the animal was walking quadrupedally (gray bar). The large increase in effectiveness was partly due to the stimulus offset occurring around the onset of the contralateral flexor burst. Thus the ipsilateral limb 'waited' until flexion was completed on the contralateral side. C. Line graph showing that the normal unperturbed cycle period increased during periods when the forelimbs were not stepping.

reported in chapters 4 and 5. The methods we followed were generally similar to those reported in chapter 4. However, additionally in these experiments we attempted to get a fictive locomotor rhythm. In the first experiment we stimulated the MLR region of the brainstem and stimulated the previously cut LGS nerve and the PL nerve from one limb with pulses of 3 stimuli at a frequency of 150 Hz and at group I strengths. The results from recording an MG motoneuron are reported in figure A2-3. As can be seen when the stimulus was off we were able to record the typical IPSPs when we stimulated the PL nerve as has been reported by Gossard et al., (1994). No IPSPs were recorded when we stimulated the LGS nerve. When the MLR was switched on, similar trains of PL stimuli produced a nice slow potential typical of stimulating the PL nerve. This can be clearly seen when the MLR off traces were subtracted from the MLR on traces. In contrast the effects of stimulating the LGS nerve did not produce an increase in the slow EPSP (monosynatpic EPSPs and possibly disynaptic EPSPs were evident). This results suggests that (1) the group I extensor afferents were still conducting and (2) the decline in amplitude of the oligosynaptic pathway was likely responsible for the effects on the amplitude of the MG EMG that we reported in chapter 5 (figure 5-3). Later we replicated these results this time using an L-DOPA preparation. As can be seen from figure A2-4, stimulation of the PL nerve similar to figure A2-3 had produced a huge oligosynaptic slowly rising EPSP. In contrast stimulation of the LGS nerve did not produce any at all.

A2-2. Increasing the speed of the treadmill decreased the effectiveness of stimulation of the LGS group I afferents in prolonging extension. Bar chart shows the percentage increase in the duration of the step cycle following stimulation of the extensor group I afferents at various treadmill speeds. The animal was bipedally stepping.

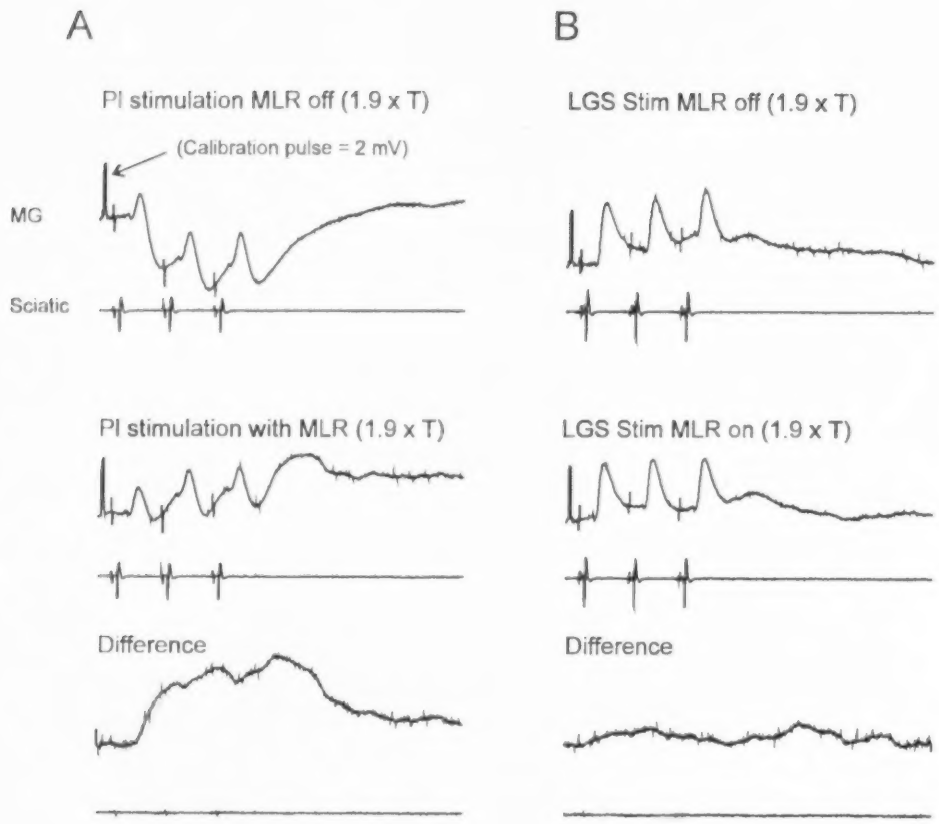

Figure A2-3: Absence of the long-latency EPSP after stimulating the LGS group I afferents. A, B. Intracellular records from the same motoneurons with the MLR on (Top Traces) and off (middle traces). The bottom traces were generated by subtracting the traces with MLR stimulation from those without MLR stimulation. A. Stimulation of PL nerve at 1.9 x T without MLR stimulation shows the presence of the IPSP potentials. When the MLR stimulus is on a long-latency potential is produced. B. In contrast stimulation of the LGS nerve produced neither a large IPSP or long-latency EPSP (when the MLR was turned on). The LGS nerve was axotomized for 17 days. Note the presence of the monosynaptic EPSPs in the MG motoneuron after stimulation of the LGS nerve.

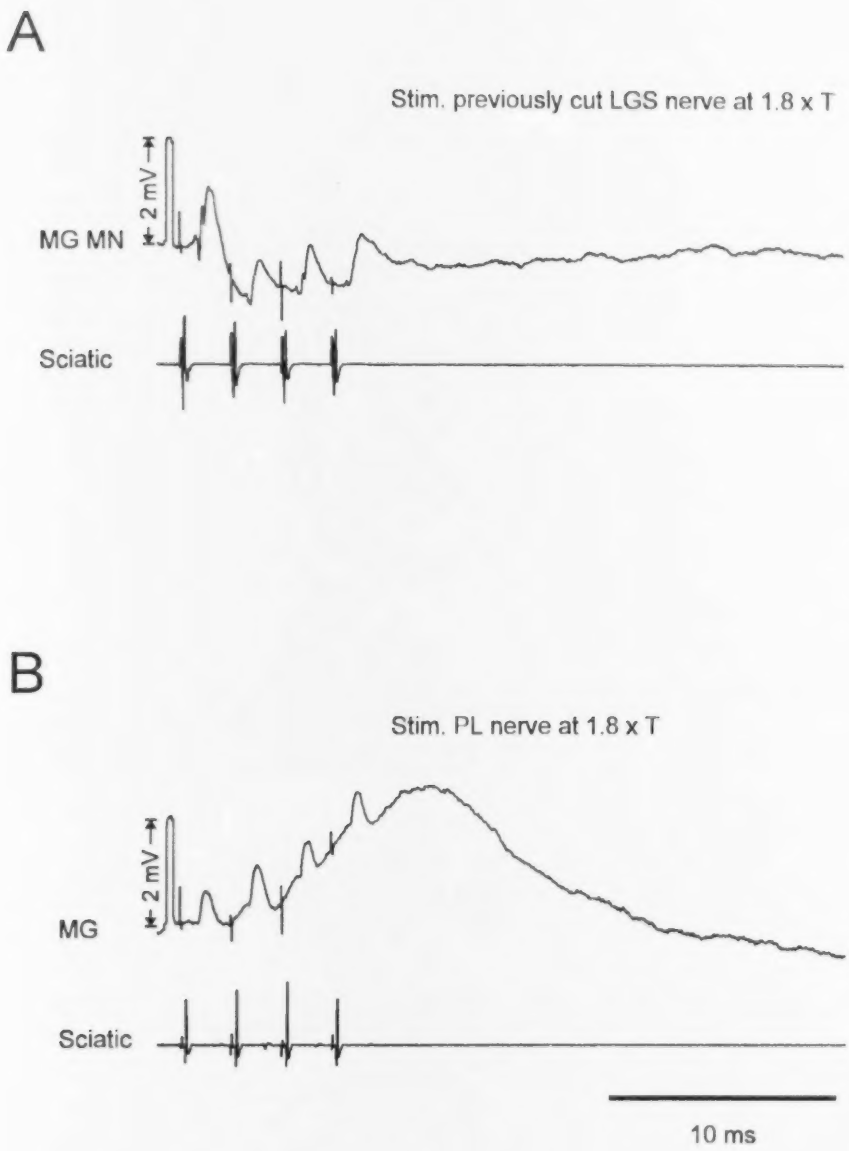

Figure A2-4 Absence of the long-latency EPSP from stimulating the LGS group I afferents after administration of L-DOPA in a spinalized animal. A, B. Intracellular records from the same MG motoneuron. A. Stimulation of the previously cut LGS nerve at 1.8 x T shows the absence of the long-latency EPSP in the MG motoneuron. B. In contrast note the large-long latency EPSP produced in the same motoneuron when the PL was stimulated. Nialamide was administered (50 mg/kg) followed by L-DOPA (50 mg/kg) to this animal. Unit was recorded approximately 2 hours after administration of L-DOPA.